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OB PROTEIN RECEPTOR AND RELATED COMPOSITIONS AND METHODS

FIELD OF THE INVENTION

The present invention relates to OB protein receptors, related compositions and methods of making and using such receptors and related compositions.

BACKGROUND

Although the molecular basis for obesity is largely unknown, the identification of the "OB gene" and protein encoded ("OB protein") has shed some light on mechanisms the body uses to regulate body fat deposition. Zhang et al., Nature 372: 425-432 (1994); see also, the Correction at Nature 374: 479 (1995). The OB protein is active in vivo in both *ob/ob* mutant mice (mice obese due to a defect in the production of the OB gene product) as well as in normal, wild type mice. The biological activity manifests itself in, among other things, weight loss. See generally, Barinaga, "Obese" Protein Slims Mice, Science 269: 475-476 (1995). See PCT International Publication Number WO 96/05309, "Modulators of Body Weight, Corresponding Nucleic Acids and Proteins, and Diagnostic and Therapeutic Uses Thereof," herein incorporated by reference.

The other biological effects of OB protein are not well characterized. It is known, for instance, that in *ob/ob* mutant mice, administration of OB protein results in a decrease in serum insulin levels, and serum glucose levels. It is also known that administration of OB protein results in a decrease in body fat. This was observed in both ob/ob mutant mice, as well as non-obese normal mice. Pelleymounter et al., Science 269: 540-543 (1995); Halaas et al., Science 269: 543-546 (1995). See

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also, Campfield et al., Science 269: 546-549 (1995) (Peripheral and central administration of microgram doses of OB protein reduced food intake and body weight of *ob/ob* and diet-induced obese mice but not in *db/db* obese mice.) In none of these reports have toxicities been observed, even at the highest doses.

Despite the promise of clinical application of the OB protein, the mode of action of the OB protein in vivo is not clearly elucidated, in part due to the absence of information on the OB receptor. High affinity binding of the OB protein has been detected in the rat hypothalamus, reportedly indicating OB receptor location. Stephens et al., Nature 377: 530-532 (1995). The *db/db* mouse displays the identical phenotype as the *ob/ob* mouse, i.e., extreme obesity and Type II diabetes; this phenotype is thought to be due to a defective OB receptor, particularly since *db/db* mice fail to respond to OB protein administration. See Stephens et al., supra.

Identification of the OB protein receptor is key in determining the pathway of signal transduction. Moreover, identification of the OB protein receptor would provide powerful application in diagnostic uses, for example, to determine if individuals would benefit from OB protein therapy. Furthermore, the OB receptor could be a key component in an assay for determining additional molecules which bind to the receptor and result in desired biological activity. Further, such soluble receptor could enhance or alter the effectiveness of OB protein (or analog or derivative thereof).

SUMMARY OF THE INVENTION

The present invention relates to a novel class of protein receptors, herein denominated "OB protein receptors" or "OB receptors", which are thought to selectively bind OB protein. As such, the novel OB

receptor family is provided, as well as novel members of such family. Also provided are nucleic acids, vectors and host cells containing such nucleic acids, related antisense nucleic acids, molecules which selectively bind to the OB protein receptor, and related compositions of matter, such as OB receptor protein/OB protein complexes. In other aspects, the present invention relates to methods of using the above compositions, such as therapeutic and/or diagnostic methods, and methods for preparing OB receptor ligands.

DETAILED DESCRIPTION

A novel family of OB receptors is provided. This novel family resulted from identification of a PCR fragment isolated from a human liver cell cDNA library. The original PCR fragment, from which primers were isolated, contained a "WSXWS" motif, common to cytokine receptors. As illustrated by the working examples below, using this fragment four members of this OB protein receptor family have been identified. These members, herein designated as "A", "B", and "C", and "D" are indentical at amino acid position 1-891 (using the numbering of Seq. ID No. 1), but diverge at position 892 through the C-terminus. They vary in length at the C-terminus beyond amino acid 891, and the different forms appear to have different tissue distribution.

Using hydrophobicity analysis, the leader sequence is likely to comprise amino acids (Seq. ID. No. 1) 1-21, 1-22, or 1-28. The first amino acid of the mature protein is likely to be 22 (F), 23 (N) or 29 (T). Most likely, based on analysis of eucaryotic cell expression (CHO cell expression see Example 8, infra), the first amino acid of the mature protein is 22(F). The beginning of the transmembrane domain appears to be located at position 840 (A) or 842 (L). The end of the transmembrane domain appears to be located at position

862 (I), 863 (S) or 864 (H). Thus, based on predictions from hydrophobicity analysis, for OB protein binding, at a minimum what is needed is the extracellular domain of the mature protein, amino acids 22, 23 or 29 through amino acids 839 (D) or 841 (G). Therefore, the present class of OB receptor proteins includes those having amino acids (according to Seq. ID No. 1):

(a) 1-896;
(b) 22-896;
(c) 23-896;
(d) 29-896;
(e) 1-839;
(f) 22-839;
(h) 1-841;
(i) 22-841;
(j) 23-841;
(k) 29-841;
(l) 1-891;
(m) 22-891;
(n) 23-891;
(o) 29-891;
(p) the amino acids of subparts (l) through (o) having the C-terminal amino acids selected from among:

(i) OB receptor B (Seq. ID No. 3) positions 892-904;

(ii) OB receptor C (Seq. ID No. 5) positions 892- 958; and,

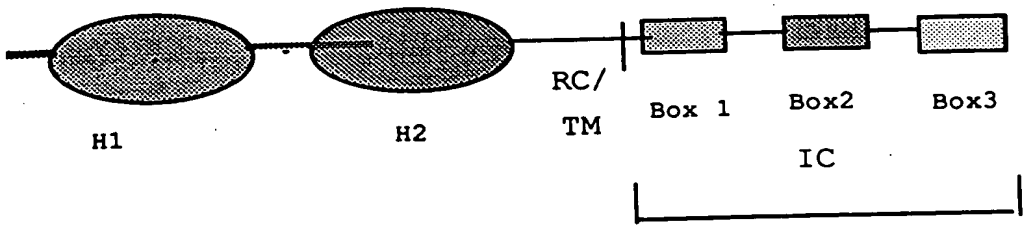
(iii) OB receptor D (Seq. ID No. 7) positions 892-1165;

(q) amino acids of subparts b, c, d, f, g, i, j, k, m, n, o, and any of (p) lacking a leader sequence, which have an N-terminal methionyl residue.

Also provided herein is what is thought to be a human splice variant of a soluble OB receptor. This

splice variant includes the extracellular domain at least up to amino acid 798 (of Seq. ID No. 1, for example) and has a unique 6 amino acid C-terminus at positions 799-804: G K F T I L.

The functional domains of the OB receptor may be predicted using the information contained in Bazan et al., PNAS-USA 87: 6934-6938 (1990) (incorporated herein by reference). For the present OB receptor, there are two hematopoietin domains, a random coil region, the transmembrane domain, and the intracellular domain. The overall geography may be illustrated as follows:



Using the information provided by Bazan, supra, the domains may be predicted, with essentially an error of approximately plus or minus three base pairs (as applied to all amino acid location specified for purposes of identifying the Bazan predicted domains). The precise locations may be determined empirically by methods known in the art, such as preparing and expressing modified recombinant DNAs. The structural characteristics are though to be important for maintaining the structural integrity of the molecule, and therefore, to the extent that such structure is important for function, for functional characteristics as well.

The hematopoietin domains (H1 and H2) are thought to have two fibronectin type 3 repeats each, one set of paired cysteine residues each (thought to form a disulfide bridge), and one "WSXWS box" (referring to the

single letter amino acid abbreviation, with "X" being any amino acid). The fibrinectin type 3 domains may be identified by location of a double proline ("PP"), which marks the beginning of the second fibronectin type 3 repeat; the actual beginning of such second fibronectin type 3 repeat is likely to begin about 3 amino acids upstream of that double proline.

The first hematopoietin domain is likely to begin at amino acid 123 (using the numbering according to Seq. ID No. 1, for example), which is an isoleucine residue (I). The last amino acid of the hematopoietin domain is likely to be amino acid 339, which is a lysine (K) residue. The two fibronectin type 3 repeats are likely to be located at (about) amino acids 123 through 235 and 236 through 339. There is a single pair of cysteine residues which likely form a disulfide bridge, located at position 131 and position 142. The "WSXWS box" is located at position 319 through 323.

The second hematopoietin domain is likely to begin at position 428, which is an isoleucine (I) and end at position 642 which is a glycine (G). The paired fibronectin type 3 repeats are located at about position 428 through position 535 and about position 536 through about position 642. One pair of cysteines is located at position 436 and position 447, and the second pair is located at position 473 and 488. The "WSXWS box" is located at position 622-626.

Between the first and the second hematopoietin domain (amino acids 339-428, approximately) is a region of unknown functional significance.

The random coil domain ("RC" between the H2 and the transmembrane domain, "TM") is likely to begin at the amino acid following the end of the second hematopoietin domain, and is likely to end at the beginning of the transmembrane domain. This is likely to be from about amino acid 642 through amino acid 839

or 841 (with the transmembrane domain beginning at position 840 (A) or 842 (L)). The intracellular domain ("IC") is likely to begin at position 861 (L), 862 (I), 863 (S) or 864 (H).

The intracellular domain ("IC") contains three regions, or "boxes," thought to participate in signal transduction (two "JAK" boxes and a single "STAT" box, "Box 1", "Box 2", and "Box 3"). With respect to the numbering of the amino acid positions of the "D" form of the OB receptor (Seq. ID No.7, below), box 1 is located at amino acid 871 (F) through 878 (P). Box 2 is located at approximately amino acid number 921 (I) through 931 (K). Box 3 on the "D" form is located at approximately position 1141 through 1144 (amino acids YMPQ, as the "STAT" box is typically a conserved region of "YXXQ" wherein "X" designates any amino acid). The intracellular domain is thought to be responsible for signal transduction. One possible mode of action is via phosphorylation of various residues. See Ihle et al., Cell 84: 331-334 (1996) (Review article, herein incorporated by reference.)

One possible mode of action is that upon ligand binding (here, OB protein binding), the OB receptor dimerizes with another receptor. A kinase ("JAK") binds to box 1, and becomes phosphorylated. (The JAK may already be bound prior to dimerization.) Also, "STATS" bind to box 3 and become phosphorylated on a specific tyrosine. It is thought that this phosphorylation results, probably indirectly, in DNA binding protein production, which results in altered DNA transcription, and therefore altered expression. As seen below in Example 6, one measurement of the capability of an OB receptor to transduce signal is the degree of phosphorylation of JAK/STAT molecules.

The C-terminus region is intracellular (of cell-bound OB receptor). The differences in the C-

terminus among members of the present OB receptor family may result in differences in signal transduction among the species. Thus, the present OB receptors include at least the extracellular domain which is important for OB protein ligand binding. Nucleic acids encoding the present OB receptors, vectors, and host cells are also provided for herein.

The extracellular domain may be modified and still retain the function of ligand binding, particularly by one or more of the following modifications: (a) the random coil domain (as indicated above, occuring downstream of the second hematopoietic domain through the beginning of the transmembrane domain) may be deleted (this may be approximately positions 642 through 839 or 841); (b) the "WSXWS" box may be modified by (i) substitution of the first serine with another amino acid, particularly conserved in terms of hydrophobicity and/or charge, such as a glycine; (ii) the last serine may be substituted with another amino acid, such as a threonine; (iii) the first tryptophan may be substituted with another amino acid, for example, a tyrosine.

Human genomic DNA encoding OB receptor protein is also provided herein. The genomic DNA has been localized to human chromosome 1P31, which is believed to correspond to mouse chromosome 4, the location of the mouse *db* locus.

Tissue distribution analysis demonstrates the presence of OB receptor nucleic acids is fairly ubiquitous, and particularly noted in the liver. It is also observed in the ovary, and heart; and, to a lesser extent, in small intestine, lung, skeletal muscle, kidney, and, to an even lesser extent, spleen, thymus, prostate, testes, placenta and pancreas (Example 2, below). There may also be one or more forms of the OB receptor present in serum, such as soluble OB receptor,

which may be complexed to one or more forms of the OB protein.

Amino Acid Sequences and Compositions

According to the present invention, novel OB protein receptors and DNA sequences coding for all or part of such OB receptors are provided. The present invention provides purified and isolated polypeptide products having part or all of the primary structural conformation (i.e., continuous sequence of amino acid residues) and one or more of the biological properties (e.g., immunological properties and in vitro biological activity) and physical properties (e.g., molecular weight) of naturally-occurring mammalian OB receptor including allelic variants thereof. The term "purified and isolated" herein means substantially free of unwanted substances so that the present polypeptides are useful for an intended purpose. For example, one may have a recombinant human OB receptor substantially free of human proteins or pathological agents. These polypeptides are also characterized by being a product of mammalian cells, or the product of chemical synthetic procedures or of procaryotic or eucaryotic host expression (e.g., by bacterial, yeast, higher plant, insect and mammalian cells in culture) of exogenous DNA sequences obtained by genomic or cDNA cloning or by gene synthesis. The products of expression in typical yeast (e.g., Saccharomyces cerevisiae), insect, or procaryote (e.g., E. coli) host cells are free of association with any mammalian proteins. The products of expression in vertebrate (e.g., non-human mammalian (e.g. COS or CHO) and avian) cells are free of association with any human proteins. Depending upon the host employed, and other factors, polypeptides of the invention may be glycosylated with mammalian or other eucaryotic carbohydrates or may be non-glycosylated. One may modify

the nucleic acid so that glycosylation sites are included in the resultant polypeptide. One may choose to partially or fully deglycosylate a glycosylated polypeptide. Polypeptides of the invention may also include an initial methionine amino acid residue (at position -1 with respect to the first amino acid residue of the mature polypeptide).

In addition to naturally-occurring allelic forms of OB receptor, the present invention also embraces other OB receptor products such as polypeptide analogs of OB receptor and fragments of OB receptor. Following the procedures of the above noted published application by Alton et al. (WO 83/04053), one can readily design and manufacture genes coding for microbial expression of polypeptides having primary conformations which differ from that herein specified for in terms of the identity or location of one or more residues (e.g., substitutions, terminal and intermediate additions and deletions). Alternately, modifications of cDNA and genomic genes may be readily accomplished by well-known site-directed mutagenesis techniques and employed to generate analogs and derivatives of OB receptor. Such products would share at least one of the biological properties of mammalian OB receptor but may differ in others. As examples, projected products of the invention include those which are foreshortened by e.g., deletions; or those which are more stable to hydrolysis (and, therefore, may have more pronounced or longer lasting effects than naturally-occurring); or which have been altered to delete one or more potential sites for glycosylation (which may result in higher activities for yeast-produced products); or which have one or more cysteine residues deleted or replaced by, e.g., alanine or serine residues and are potentially more easily isolated in active form from microbial systems; or which have one or more tyrosine residues

replaced by phenylalanine; or have an altered lysine composition (such as those prepared for purposes of derivatization). Included are those polypeptides with amino acid substitutions which are "conservative" according to acidity, charge, hydrophobicity, polarity, size or any other characteristic known to those skilled in the art. See generally, Creighton, Proteins, W.H. Freeman and Company, N.Y., (1984) 498 pp. plus index, passim. One may make changes in selected amino acids so long as such changes preserve the overall folding or activity of the protein, (see Table 1, below). Small amino terminal extensions, such as an amino-terminal methionine residue, a small linker peptide of up to about 20-25 residues, or a small extension that facilitates purification, such as a poly-histidine tract, an antigenic epitope or a binding domain, may also be present. See, in general Ford et al., Protein Expression and Purification 2: 95-107, 1991, which is herein incorporated by reference.

Table 1
Conservative Amino Acid Substitutions

Basic:	arginine lysine histidine
Acidic:	glutamic acid aspartic acid
Polar:	glutamine asparagine
Hydrophobic:	leucine isoleucine valine
Aromatic:	phenylalanine tryptophan tyrosine
Small:	glycine alanine serine threonine methionine

Also comprehended are polypeptide fragments duplicating only a part of the continuous amino acid sequence or secondary conformations within OB receptor, which fragments may possess one activity of mammalian (particularly human) OB receptor (e.g., immunological activity) and not others (e.g., OB protein binding activity).

Of applicability to OB receptor fragments and polypeptide analogs of the invention are reports of the immunological activity of synthetic peptides which substantially duplicate the amino acid sequence extant in naturally-occurring proteins, glycoproteins and nucleoproteins. More specifically, relatively low

molecular weight polypeptides have been shown to participate in immune reactions which are similar in duration and extent to the immune reactions of physiologically significant proteins such as viral antigens, polypeptide hormones, and the like. Included among the immune reactions of such polypeptides is the provocation of the formation of specific antibodies in immunologically active animals. See, e.g., Lerner et al., Cell 23: 309-310 (1891); Ross et al., Nature 294: 654-656 (1891); Walter et al., PNAS-USA 77: 5197-5200 (1980); Lerner et al., PNAS-USA, 78: 3403-3407 (1891); Walter et al., PNAS-USA 78: 4882-4886 (1891); Wong et al., PNAS-USA 79: 5322-5326 (1982); Baron et al., Cell 28: 395-404 (1982); Dressman et al., Nature 295: 185-160 (1982); and Lerner, Scientific American 248: 66-74 (1983). See, also, Kaiser et al. Science 223: 249-255 (1984) relating to biological and immunological activities of synthetic peptides which approximately share secondary structures of peptide hormones but may not share their primary structural conformation. The present invention also includes that class of polypeptides coded for by portions of the DNA complementary to the protein-coding strand of the human cDNA or genomic DNA sequences of OB receptor i.e., "complementary inverted proteins" as described by Tramontano et al. Nucleic Acid Res. 12: 5049-5059 (1984). Polypeptides or analogs thereof may also contain one or more amino acid analogs, such as peptidomimetics.

Thus, the present class of OB receptor proteins includes those having amino acids (according to Seq. ID No. 1):

(a) 1-896;
(b) 22-896;
(c) 23-896;
(d) 29-896
(e) 1-839;

(f) 22-839;
(g) 29-839;
(h) 1-841;
(i) 22-841;
(j) 23-841;
(k) 29-841;
(l) 1-891;
(m) 22-891;
(n) 23-891;
(o) 29-891;
(p) the amino acids of subparts (l) through (o) having the C-terminal amino acid sequence beginning at position 892 of OB receptor B (Seq. ID No. 3) or C (Seq. ID. No. 5);
(q) amino acids of subparts b, c, d, f, g, i, j, k, m, n, o, and any of (p) lacking a leader sequence, which have an N-terminal methionyl residue.

Also provided is a longer form of an OB receptor protein, herein denominated the "D" form, which has an amino acid sequence selected from among (according to Seq. ID No. 7):

(a) amino acids 1-1165;
(b) amino acids 22-1165;
(c) amino acids 23-1165;
(d) amino acids 29-1165;
(e) amino acids of subparts (b), (c) or (d) having an N-terminal methionyl residue.

As set forth above, one may prepare soluble receptor by elimination of the transmembrane and intracellular regions. Examples of soluble receptors include those set forth in Seq. ID Nos. 10 and 13. What is thought to be a native, secreted form of a soluble human OB receptor is also provided herein. This form of OB receptor protein has an amino acid sequence selected from among (according to Seq. ID No. 13):

(a) amino acids 1-804;

(b) amino acids 22-804;

(c) amino acids 23-804;

(d) amino acids 29-804; and,

(e) amino acids of subparts (b), (c) or (d) having an N-terminal methionyl residue.

In addition, since the C-terminus region of the above polyeptides diverges at position 892 (with respect to Seq. ID Nos. 1, 3, 5, 7 and 13) one may desire to prepare only the polypeptides which are divergent:

(a) those having only amino acids 892-896 of Seq. ID No. 1;

(b) those having only amino acids 892-904 of Seq. ID No. 3;

(c) those having only amino acids 892-958 of Seq. ID No. 5;

(d) those having only amino acids 892-1165 of Seq. ID No. 7; and,

(e) those having only amino acids 799-804 of Seq. ID No. 13.

The above polypeptides which have an extracellular domain may be modified, as indicated above, and still retain the function of ligand binding. Such modification may include one or more of the following:

(a) the random coil domain (as indicated above, occuring downstream of the second hematopoietic domain through the beginning of the transmembrane domain) may be deleted (this may be approximately positions 642 through 839 or 841);

(b) the "WSXWS" box may be modified by (i) substitution of the first serine with another amino acid, particularly conserved in terms of hydrophobicity and/or charge, such as a glycine; (ii) the last serine may be substituted with another amino acid, such as a threonine; (iii) the first tryptophan may be

substituted with another amino acid, for example, a tyrosine.

Thus, the present polypeptides include (according to the numbering of Seq. ID No. 7):

(a) 1-896;

(b) 22-896;

(c) 23-896;

(d) 29-896

(e) 1-839;

(f) 22-839;

(g) 29-839;

(h) 1-841;

(i) 22-841;

(j) 23-841;

(k) 29-841;

(l) 1-891;

(m) 22-891;

(n) 23-891;

(o) 29-891;

(p) the amino acids of subparts (l) through (o) having the C-terminal amino acids selected from the C-terminal amino acids of OB receptor B (Seq. ID No. 3), C (Seq. ID. No. 5) and D (Seq ID No. 7);

(q) the amino acids (according to Seq. ID No. 13) selected from the group consisting of 22-804; 23-804 and 29-804;

(r) amino acids of subparts b, c, d, f, g, i, j, k, m, n, o, any of (p) lacking a leader sequence, and (q) which have an N-terminal methionyl residue; and

(s) amino acids of subparts (a) through (r) which above having at least one of the following modifications:

(i) for amino acids of subparts (a) through (p) and those of subpart (r) which are not amino acids according to subpart (q), deletion of (or

substitution of amino acid(s) or other modifications of) a random coil domain sequence selected from

(a) 640 through 839 (using the numbering according to Seq. ID No. 1);

(b) 641 through 839;

(c) 642 through 839;

(d) 640 through 841;

(e) 641 through 841; and

(f) 642 through 841;

(ii) for amino acids of subpart (q) and those of subpart (r) which contain the sequence of subpart (q), deletion of of (or substitution of amino acid(s) or other modifications of) a random coil domain sequence selected from among:

(a) 640 through 804;

(b) 641 through 804; and,

(c) 642 through 804;

and,

(iii) modification of a "WSXWS" sequence which is

(a) substitution of the first serine with another amino acid, particularly conserved in terms of hydrophobicity and/or charge, such as a glycine;

(b) substition of the last serine with another amino acid, such as a threonine; and

(c) substitution of the first tryptophan with another amino acid, for example, a tyrosine.

One may modify the OB receptor to create a fusion molecule with other peptide sequence. For example, if one desired to "tag" the OB receptor with an immunogenic peptide, one could construct a DNA which would result in such fusion protein. The tag may be at the N-terminus. Also, since it is apparent that the

C-terminus is not necessary for ligand binding activity, one may chemically modify the C-terminus of, for example, a soluble OB receptor. One may desire, for example, a preparation whereby one or more polymer molecules such as polyethylene glycol molecules are attached. Thus, another aspect of the present invention is chemically modified OB receptor protein (also further described infra).

An example of such "tag" is provided herein using the C-terminus of a recombinant soluble OB receptor. Seq. ID No. 12 provides a "FLAG-tag" version of such soluble OB receptor (the nucleic acid sequence is provided, which may be transcribed to prepare the polypeptide). Such "FLAG-tag" may also be attached to the N-terminus or other region of an OB receptor protein. This type of "tagging" is useful to bind the protein using reagents, such as antibodies, which are selective for such tag. Such binding may be for detection of the location or amount of protein, or for protein capturing processes where, for example, an affinity column is used to bind the tag, and thus the desired protein. Other types of detectable labels, such as radioisotopes, light-emitting (e.g., fluorescent or phosporescent compounds), enzymatically cleavable, detectable antibody (or modification thereof), or other substances may be used for such labelling of the present proteins. Detecting protein via use of the labels may be useful for identifying the presence or amount of OB receptor protein or a compound containing such protein (e.g., OB protein complexed to OB receptor). Moreover, such labelled protein may be useful for distinguishing exogenous OB receptor protein from the endogenous form.

Nucleic Acids

Novel nucleic acid sequences of the invention include sequences useful in securing expression in procaryotic or eucaryotic host cells of polypeptide products having at least a part of the primary structural conformation and one or more of the biological properties of recombinant human OB receptor. The nucleic acids may be purified and isolated, so that the desired coding region is useful to produce the present polypeptides, for example, or for diagnostic purposes, as described more fully below. DNA sequences of the invention specifically comprise: (a) any of the DNA sequences set forth in Seq. ID No. 2, 4, 6, 8, 9, 11, 12, and 14 (and complementary strands); (b) a DNA sequence which hybridizes (under hybridization conditions disclosed in the cDNA library screening section below, using the 300 bp PCR fragment as described to selectively hybridize to a cDNA encoding an OB receptor protein in a human liver cDNA library, or equivalent conditions or more stringent conditions) to the DNA sequence in subpart (a) or to fragments thereof; and (c) a DNA sequence which, but for the degeneracy of the genetic code, would hybridize to the DNA sequence in subpart (a). Specifically comprehended in parts (b) and (c) are genomic DNA sequences encoding allelic variant forms of human OB receptor and/or encoding OB receptor from other mammalian species, and manufactured DNA sequences encoding OB receptor, fragments of OB receptor, and analogs of OB receptor which DNA sequences may incorporate codons facilitating transcription and translation of messenger RNA in microbial hosts.. Such manufactured sequences may readily be constructed according to the methods of Alton et al., PCT published application WO 83/04053.

Genomic DNA, such as that of Seq. ID No. 9, encoding the present OB receptors may contain additional non-coding bases, or introns, and such genomic DNAs are obtainable by hybridizing all or part of the cDNA, illustrated in Seq. ID Nos. 2, 4, 6, 8, 11, and 14 to a genomic DNA source, such as a human genomic DNA library. Such genomic DNA will encode functional OB receptor polypeptide; however, use of the cDNAs may be more practicable in that, since only the coding region is involved, recombinant manipulation is facilitated. The intron/exon location of genomic DNA is set forth in Seq. ID No. 9, infra.

Nucleic acid sequences include the incorporation of codons which enhance expression by selected nonmammalian hosts; the provision of sites for cleavage by restriction endonuclease enzymes; and the provision of additional initial, terminal or intermediate DNA sequences which facilitate construction of cloning and/or expression vectors.

The present invention also provides DNA sequences coding for polypeptide analogs or derivatives of OB receptor which differ from naturally-occurring forms in terms as described above. The leader sequence DNA may be substituted with another leader sequence for ease in expression or for other purposes.

Also, one may prepare antisense nucleic acids against the present DNAs. Such antisense nucleic acids may be useful in modulating the effects of OB receptor protein in vivo. For example, one may prepare an antisense nucleic acid which effectively disables the ability of a cell to produce OB receptor by binding to the nucleic acid which encodes such OB receptor.

DNA sequences of the invention are also suitable materials for use as labeled probes in isolating human genomic DNA encoding OB receptor, as mentioned above, and related proteins as well as cDNA

and genomic DNA sequences of other mammalian species. DNA sequences may also be useful in various alternative methods of protein synthesis (e.g., in insect cells) or, as described infra, in genetic therapy in humans and other mammals. DNA sequences of the invention are expected to be useful in developing transgenic mammalian species which may serve as eucaryotic "hosts" for production of OB receptor and OB receptor products in quantity. See, generally, Palmiter et al., Science 222: 809-814 (1983).

Vectors and Host Cells

According to another aspect of the present invention, the DNA sequences described herein which encode OB receptor polypeptides are valuable for the information which they provide concerning the amino acid sequence of the mammalian protein which have heretofore been unavailable. Put another way, DNA sequences provided by the invention are useful in generating new and useful viral and circular plasmid DNA vectors, new and useful transformed and transfected procaryotic and eucaryotic host cells (including bacterial cells, yeast cells, insect cells, and mammalian cells grown in culture), and new and useful methods for cultured growth of such host cells capable of expression of OB receptor and its related products.

The DNA provided herein (or corresponding RNAs) may also be used for gene therapy for, example, treatment of conditions characterized by the overexpression of OB protein, such as anorexia or cachexia. Alternatively, gene therapy may be used in cases where increased sensitivity to OB protein is desired, such as in cases where an individual has a condition characterized by OB protein receptors defective in ability to bind or retain the binding of OB protein. Currently, vectors suitable for gene therapy

(such as retroviral or adenoviral vectors modified for gene therapy purposes and of purity and pharmaceutical acceptability) may be administered for delivery into the lung, for example. Such vectors may incorporate nucleic acid encoding the present polypeptides for expression in a desired location. Gene therapy may involve more than one gene for a desired protein or different desired proteins.

Alternatively, one may use no vector so as to facilitate relatively stable presence in the host. For example, homologous recombination of a DNA as provided herein or of a suitable transcription or translation control region may facilitate integration into or expression from a host genome. (This may be performed for production purposes as well, e.g., U.S. Patent No. 5,272,071 and WO 91/09955.) The nucleic acid may be placed within a pharmaceutically acceptable carrier to facilitate cellular uptake, such as a lipid solution carrier (e.g., a charged lipid), a liposome, or polypeptide carrier (e.g., polylysine). A review article on gene therapy is Verma, Scientific American, November 1990, pages 68-84 which is herein incorporated by reference.

Thus, the present invention provides for a population of cells expressing an OB receptor of the present OB receptor family. Such cells are suitable for transplantation or implantation into an individual for therapeutic purposes. For example, one may prepare a population of cells to overexpress OB receptor (such as one identified in the Sequence ID's or otherwise denoted herein), or to express a desired form of OB receptor, such as one which is particularly sensitive to OB protein (i.e., a form which has a desired capacity for signal transduction). One may then implant such cells into an individual to increase that individual's sensitivity to OB protein. Such cells may, for example,

be liver cells, bone marrow cells, or cells derived from umbillical cord. Alternatively, one may wish to use overexpressing circulating cells such as blood progenitor cells, T cells or other blood cells. For humans, human cells may be used. Cells may be in the form of tissue. Such cells may be cultured prior to transplantation or implantation. Such OB receptor overexpression, or expression of particularly sensitive forms of OB receptor may be accomplished by, for example, altering the regulatory mechanism for expression of OB receptor, such as using homologous recombination techniques as described supra. Thus, provided is a population of host cells modified so that expression of endogenous OB receptor DNA is enhanced.

The cells to be transferred to the recipient may be cultured using one or more factors affecting the growth or proliferation of such cells if appropriate. Hematopoietic factors may be used in culturing hematopoietic cells. Such factors include G-CSF, EPO, MGDF, SCF, Flt-3 ligand, interleukins (e.g., IL1-IL13), GM-CSF, LIF, and analogs and derivatives thereof as available to one skilled in the art.

Nerve cells, such as neurons or glia, may also be used, and these may be cultured with neurotrophic factors such as BDNF, CNTF, GDNF, NT3, or others.

There may be a co-gene therapy involving the transplantation of cells expressing more than one desired protein. For example, cells expressing OB receptor protein may be used in conjunction, simultaneously or in serriatim with cells expressing OB protein.

For gene therapy dosages, one will generally use between one copy and several thousand copies of the present nucleic acid per cell, depending on the vector, the expression system, the age, weight and condition of the recipient and other factors which will be apparent

to those skilled in the art. The cellular delivery of such protein may be designed to last for a selected period of time, such as a period of days, weeks, months or years. At the end of the effective time period, the recipient of such transformed cells may receive another "dose" (e.g., transplantation of cells). Cells may be selected for their lifespan, their time period of expression of the desired protein, or their ability to be reisolated from an individual (i.e., for blood cells, leukaphoresis may be used to retrieve transformed cells using markers present on the cell surface). Vectors may be similiarly designed using, for example, viruses which have a known period of expression of DNAs contained therein.

The desired cells or vectors may be stored using techniques, such as freezing, available to those in the art.

Thus, the present invention also contemplates a method for administering OB receptor protein to an individual, wherein the source of said OB receptor protein is selected from (i) a population of cells expressing OB receptor protein and (ii) a population of vectors expressing OB receptor protein. Said OB receptor protein may be selected from among those described herein. Said vectors may be virus vectors capable of infecting human cells. Said cells may be selected from among tissue or individual cells. Said individual cells may be selected from among adipocytes, fibroblasts, bone marrow cells, peripheral blood progenitor cells, red blood cells, and white blood cells, including T cells and nerve cells. Said population of cells or vectors may be co-administered with a population of cells or vectors which express OB protein or another desired protein. Said cells or vectors may be stored for use in an individual. Storage may be by freezing

Complexes

In addition to the OB receptor protein as described herein, one may prepare complexes of OB receptor protein and OB protein, analog or derivative.

The OB protein may be selected from those described in PCT publication WO 96/05309, above and hereby incorporated by reference in its entirety. Figure 3 of that publication (Seq. ID No. 4, as cited therein) depicts the full deduced amino acid sequence derived for the human OB gene. The amino acids are numbered from 1 to 167. A signal sequence cleavage site is located after amino acid 21 (Ala) so that the mature protein extends from amino acid 22 (Val) to amino acid 167 (Cys). For the present disclosure, a different numbering is used herein, where the amino acid position 1 is the Valine residue which is at the beginning of the mature protein.

Generally, the OB protein for use will be capable of complexing to the OB protein receptor selected. Thus, one may empirically test the binding capability (to all or part of the extracellular domain of the OB receptor as indicated above) to determine which OB protein forms may be used. Generally, modifications generally applicable as indicated above for OB receptor protein may also be applied here, and that disclosure is incorporated by reference here. As set forth in WO 96 05309, OB protein in its native form, or fragments (such as enzyme cleavage products) or other truncated forms, analogs, and derivatives all retain biological activity. Such forms may be used so long as the form binds to at least a portion of the extracellular domain of the present OB receptor proteins.

An effective amount of an OB protein, analog or derivative thereof may be selected from among

according to the amino acid sequence as presented in PCT WO 96/05309, Figure 3 numbered so that the first amino acid of the mature protein is number 1:

(a) the amino acid sequence 1-146, optionally lacking a glutaminyl residue at position 28, and further optionally having a methionyl residue at the N-terminus;

(b) an amino acid sequence of subpart (a) having a different amino acid substituted in one or more of the following positions: 4, 8, 32, 33, 35, 48, 50, 53, 60, 64, 66, 67, 68, 71, 74, 77, 78, 89, 97, 100, 102, 105, 106, 107, 108, 111, 112, 118, 136, 138, 142, and 145;

(c) a truncated OB protein analog selected from among: (using the numbering of subpart (a) above):

(i) amino acids 98-146
(ii) amino acids 1-32
(iii) amino acids 1-35
(iv) amino acids 40-116
(v) amino acids 1-99 and 112-146
(vi) amino acids 1-99 and 112-146 having one or more of amino acids 100-111 sequentially placed between amino acids 99 and 112; and,

(vii) the truncated OB analog of subpart (i) having one or more of amino acids 100, 102, 105, 106, 107, 108, 111, 112, 118, 136, 138, 142, and 145 substituted with another amino acid;

(viii) the truncated analog of subpart (ii) having one or more of amino acids 4, 8 and 32 substituted with another amino acid;

(ix) the truncated analog of subpart (iv) having one or more of amino acids 50, 53, 60, 64, 66, 67, 68, 71, 74, 77, 78, 89, 97, 100, 102,

105, 106, 107, 108, 111 and 112 replaced with another amino acid;

(x) the truncated analog of subpart (v) having one or more of amino acids 4, 8, 32, 33, 35, 48, 50, 53, 60, 64, 66, 67, 68, 71, 74, 77, 78, 89, 97, 112, 118, 136, 138, 142, and 145 replaced with another amino acid;

(xi) the truncated analog of subpart (vi) having one or more of amino acids 4, 8,32, 33, 35, 48, 50, 53, 60, 64, 66, 67, 68, 71, 74, 77, 78, 89, 97, 100, 102, 105, 106, 107, 108, 111, 112, 118, 136, 138, 142, and 145 replaced with another amino acid;

(xii) the truncated analog of any of subparts (i)-(xi) having an N-terminal methionyl residue; and

(d) the OB protein or analog derivative of any of subparts (a) through (c) comprised of a chemical moiety connected to the protein moiety;

(e) a derivative of subpart (d) wherein said chemical moiety is a water soluble polymer moiety;

(f) a derivative of subpart (e) wherein said water soluble polymer moiety is polyethylene glycol;

(g) A derivative of subpart (f) wherein said water soluble polymer moiety is a polyamino acid moiety;

(h) a derivative of subpart (g) wherein said water soluble polymer moiety is attached at solely the N-terminus of said protein moiety;

(i) an OB protein, analog or derivative of any of subparts (a) through (h) in a pharmaceutically acceptable carrier.

OB proteins, analogs and related molecules are also reported in the following publications; however, no

representation is made with regard to the activity of any composition reported:

U.S. Patent Nos. 5,521,283; 5,532,336; 5,552,522; 5,552,523; 5,552,524; 5,554,727; 5,559,208; 5,563,243; 5,563,244; 5,563,245; 5,567,678; 5,567,803; 5,569,744; 5,569,743 (all assigned to Eli Lilly and Company);

PCT WO96/23517; WO96/23515; WO96/23514; WO96/24670; WO96/23513; WO96/23516; WO96/23518; WO96/23519; WO96/23520; WO96/23815; WO96/24670; WO96/27385 (all assigned to Eli Lilly and Company);

PCT WO96/22308 (assigned to Zymogenetics);

PCT WO96/29405 (assigned to Ligand Pharmaceuticals, Inc.);

PCT WO96/31526 (assigned to Amyin Pharmaceuticals, Inc.);

PCT WO96/34885 (assigned to Smithkline Beecham PLC);

PCT WO96/35787 (assigned to Chiron);

EP 0 725 079 (assigned to Eli Lilly and Company);

EP 0 725 078 (assigned to Eli Lilly and Company);

EP 0 736 599 (assigned to Takeda);

EP 0 741 187 (assigned to F. Hoffman LaRoche).

To the extent these references provide for useful OB proteins or analogs or derivatives thereof, or associated compositions or methods, such compositions and/or methods may be used in conjunction with the present OB receptor proteins, such as for co-administration (together or separately, in a selected dosage schedule) or by complexing compositions to the present OB protein receptors. With the above provisos, these publications are herein incorporated by reference.

Derivatives and Formulations

The present OB protein receptor and/or OB protein (herein the term "protein" is used to include "peptide" and OB protein or receptor analogs, such as those recited infra, unless otherwise indicated) may also be derivatized by the attachment of one or more chemical moieties to the protein moiety. If the present pharmaceutical compositions contain as the active ingredient a complex of OB protein receptor and OB protein, one or both of such proteins may be derivatized. The chemically modified derivatives may be further formulated for intraarterial, intraperitoneal, intramuscular, subcutaneous, intravenous, oral, nasal, pulmonary, topical or other routes of administration. Chemical modification of biologically active proteins has been found to provide additional advantages under certain circumstances, such as increasing the stability and circulation time of the therapeutic protein and decreasing immunogenicity. See U.S. Patent No. 4,179,337, Davis et al., issued December 18, 1979. For a review, see Abuchowski et al., in Enzymes as Drugs. (J.S. Holcerberg and J. Roberts, eds. pp. 367-383 (1891)). A review article describing protein modification and fusion proteins is Francis, Focus on Growth Factors 3: 4-10 (May 1992) (published by Mediscript, Mountview Court, Friern Barnet Lane, London N20, OLD, UK).

Preferably, for therapeutic use of the end-product preparation, the chemical moiety for derivatization will be pharmaceutically acceptable. A polymer may be used. One skilled in the art will be able to select the desired polymer based on such considerations as whether the polymer/protein conjugate will be used therapeutically, and if so, the desired dosage, circulation time, resistance to proteolysis, and

other considerations. For the present proteins and peptides, the effectiveness of the derivatization may be ascertained by administering the derivative, in the desired form (i.e., by osmotic pump, or by injection or infusion, or, further formulated for oral, pulmonary or nasal delivery, for example), and observing biological effects as described herein.

The chemical moieties suitable for derivatization may be selected from among various water soluble polymers. The polymer selected should be water soluble so that the protein to which it is attached so that it is miscible in an aqueous environment, such as a physiological environment. The water soluble polymer may be selected from the group consisting of, for example, polyethylene glycol, copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random or non-random copolymers (see supra regarding fusion molecules), and dextran or poly(n-vinyl pyrolidone)polyethylene glycol, propylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols, polystyrenemaleate and polyvinyl alcohol. Polyethylene glycol propionaldenhyde may have advantages in manufacturing due to its stability in water.

Fusion proteins may be prepared by attaching polyaminoacids to the OB protein receptor or OB protein (or analog or complex) moiety. For example, the polyamino acid may be a carrier protein which serves to increase the circulation half life of the protein. For the present therapeutic or cosmetic purposes, such polyamino acid should be those which do not create neutralizing antigenic response, or other adverse response. Such polyamino acid may be selected from the

group consisting of serum album (such as human serum albumin), an antibody or portion thereof (such as an antibody constant region, sometimes called "F_C") or other polyamino acids. As indicated below, the location of attachment of the polyamino acid may be at the N-terminus of the OB protein moiety, or other place, and also may be connected by a chemical "linker" moiety to the OB protein.

The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 2 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog).

The number of polymer molecules so attached may vary, and one skilled in the art will be able to ascertain the effect on function. One may mono-derivatize, or may provide for a di-, tri-, tetra- or some combination of derivatization, with the same or different chemical moieties (e.g., polymers, such as different weights of polyethylene glycols). The proportion of polymer molecules to protein (or peptide) molecules will vary, as will their concentrations in the reaction mixture. In general, the optimum ratio (in terms of efficiency of reaction in that there is no excess unreacted protein or polymer) will be determined by factors such as the desired degree of derivatization (e.g., mono, di-, tri-, etc.), the molecular weight of

the polymer selected, whether the polymer is branched or unbranched, and the reaction conditions.

The chemical moieties should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art. E.g., EP 0 401 384 herein incorporated by reference (coupling PEG to G-CSF), see also Malik et al., Exp. Hematol. 20: 1028-1035 (1992) (reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule (or other chemical moiety) may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residue. Those having a free carboxyl group may include aspartic acid residues, glutamic acid residues, and the C-terminal amino acid residue. Sulfhydrl groups may also be used as a reactive group for attaching the polyethylene glycol molecule(s) (or other chemical moiety). Preferred for therapeutic manufacturing purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group. Attachment at residues important for receptor binding should be avoided if receptor binding is desired.

One may specifically desire N-terminally chemically modified protein. Using polyethylene glycol as an illustration of the present compositions, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining

the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective N-terminal chemical modification may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. See PCT WO 96/11953, herein incorporated by reference. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved. For example, one may selectively N-terminally pegylate the protein by performing the reaction at a pH which allows one to take advantage of the pKa differences between the e-amino group of the lysine residues and that of the a-amino group of the N-terminal residue of the protein. By such selective derivatization, attachment of a polymer to a protein is controlled: the conjugation with the polymer takes place predominantly at the N-terminus of the protein and no significant modification of other reactive groups, such as the lysine side chain amino groups, occurs. Using reductive alkylation, the polymer may be of the type described above, and should have a single reactive aldehyde for coupling to the protein. Polyethylene glycol propionaldehyde, containing a single reactive aldehyde, may be used.

An N-terminally chemically modified derivative is preferred (over other forms of chemical modification) for ease in production of a therapeutic. N-terminal chemical modification ensures a homogenous product as characterization of the product is simplified relative to di-, tri- or other multi-derivatized products. The use of the above reductive alkylation process for

preparation of an N-terminally chemically modified product is preferred for ease in commercial manufacturing.

In yet another aspect of the present invention, provided are methods of using pharmaceutical compositions of the proteins, and derivatives. Such pharmaceutical compositions may be for administration by injection, or for oral, pulmonary, nasal, transdermal or other forms of administration. In general, comprehended by the invention are pharmaceutical compositions comprising effective amounts of protein or derivative products of the invention together with pharmaceutically acceptable diluents, preservatives, solubilizers, emulsifiers, adjuvants and/or carriers. Such compositions include diluents of various buffer content (e.g., Tris-HCl, acetate, phosphate), pH and ionic strength; additives such as detergents and solubilizing agents (e.g., Tween 80, Polysorbate 80), anti-oxidants (e.g., ascorbic acid, sodium metabisulfite), preservatives (e.g., Thimersol, benzyl alcohol) and bulking substances (e.g., lactose, mannitol); incorporation of the material into particulate preparations of polymeric compounds such as polylactic acid, polyglycolic acid, etc. or into liposomes. See, e.g., PCT WO96/29989, Collins et al., "Stable protein: phospholipid compositions and methods," published October 3, 1996, herein incorporated by reference. Hylauronic acid may also be used, and this may have the effect of promoting sustained duration in the circulation. Such compositions may influence the physical state, stability, rate of in vivo release, and rate of in vivo clearance of the present proteins and derivatives. See, e.g., Remington's Pharmaceutical Sciences, 18th Ed. (1990, Mack Publishing Co., Easton, PA 18042) pages 1435-1712 which are herein incorporated by reference. The compositions may be prepared in

liquid form, or may be in dried powder, such as lyophilized form. Implantable sustained release formulations are also contemplated, as are transdermal formulations.

Specifically contemplated are oral dosage forms of the above derivatized proteins. Protein may be chemically modified so that oral delivery of the derivative is efficacious. Generally, the chemical modification contemplated is the attachment of at least one moiety to the protein (or peptide) molecule itself, where said moiety permits (a) inhibition of proteolysis; and (b) uptake into the blood stream from the stomach or intestine. Also desired is the increase in overall stability of the protein and increase in circulation time in the body. See PCT WO95/21629, Habberfield, "Oral Delivery of Chemically Modified Proteins" (published August 17, 1995) herein incorporated by reference, and U.S. Patent No. 5,574,018, Habberfield et al., "Conjugates of Vitamin B12 and Proteins," issued November 12, 1996, herein incorporated by reference.

Also contemplated herein is pulmonary delivery of the present protein, or derivative thereof. The protein (derivative) is delivered to the lungs of a mammal while inhaling and traverses across the lung epithelial lining to the blood stream. See, PCT WO94/20069, Niven et al., "Pulmonary administration of granulocyte colony stimulating factor," published September 15, 1994, herein incorporated by reference.

Nasal delivery of the protein (or analog or derivative) is also contemplated. Nasal delivery allows the passage of the protein to the blood stream directly after administering the therapeutic product to the nose, without the necessity for deposition of the product in the lung. Formulations for nasal delivery include those with absorption enhancing agents, such as dextran or

cyclodextran. Delivery via transport across other mucous membranes is also contemplated.

Dosages

One skilled in the art will be able to ascertain effective dosages by administration and observing the desired therapeutic effect. Preferably, the formulation of the molecule or complex in a pharmaceutical composition will be such that between about .10 µg/kg/day and 10 mg/kg/day will yield the desired therapeutic effect. The effective dosages may be determined using diagnostic tools over time. For example, a diagnostic for measuring the amount of OB protein or OB receptor protein in the blood (or plasma or serum) may first be used to determine endogenous levels of OB protein (or receptor). Such diagnostic tool may be in the form of an antibody assay, such as an antibody sandwich assay. The amount of endogenous OB receptor protein (such as soluble receptor) is quantified initially, and a baseline is determined. The therapeutic dosages are determined as the quantification of endogenous and exogenous OB receptor protein (that is, protein, analog or derivative found within the body, either self-produced or administered) is continued over the course of therapy. The dosages may therefore vary over the course of therapy, with a relatively high dosage being used initially, until therapeutic benefit is seen, and lower dosages used to maintain the therapeutic benefits.

During an initial course of therapy of an obese person, dosages may be administered whereby weight loss and concomitant fat tissue decrease increase is achieved. Once sufficient weight loss is achieved, a dosage sufficient to prevent re-gaining weight, yet sufficient to maintain desired weight or fat mass may be administered. These dosages can be determined

empirically, as the effects of OB protein are reversible. E.g., Campfield et al., Science 269: 546-549 (1995) at 547. Thus, if a dosage resulting in weight loss is observed when weight loss is not desired, one would administer a lower dose, yet maintain the desired weight.

Therapeutic Compositions and Methods

The present OB receptor proteins, alone, or in combination with an OB protein, and nucleic acids may be used for methods of treatment, or for methods of manufacturing medicaments for treatment. Such treatment includes conditions characterized by excessive production of OB protein, wherein the present OB receptors, particularly in soluble form, may be used to complex to and therefore inactivate such excessive OB protein. Or, such OB receptor protein, particularly in soluble form, may act to protect the activity of OB protein. While not wishing to be bound by theory, one may postulate that OB protein receptor agonist activity may be accomplished by a protective effect achieved when OB protein receptor (particularly soluble receptor) is complexed to OB protein. Such effect may prolong the serum half life of OB protein in vivo. Such treatments may be accomplished by preparing soluble receptor (e.g., use of an extracellular domain as described supra) and administering such composition to an individual in need thereof or by preparation of a population of cells containing or expressing such OB receptor, and transplanting such cells into the individual in need thereof.

The present OB receptors may also be used for treatment of those having defective OB receptors. For example, one may treat an individual having defective OB receptors by preparation of a population of cells containing such non-defective OB receptor, and

transplanting such cells into an individual. Or, an individual may have an inadequate number of OB receptors, and cells containing such receptors may be transplanted in order to increase the number of OB receptors available to an individual.

The present OB receptor proteins and related compositions such as OB receptor protein/OB protein complex, provide for weight loss, fat loss, increase in lean mass, increase in insulin sensitivity, increase in overall strength, increase in red blood cells (and oxygenation in the blood), decrease in bone resportion or osteoporosis, decreased or maintained serum cholesterol level, decreased or maintained triglyceride (LDL or VLDL) levels, prevention or reduction in arterial plaque formation, treatment of hypertension, and prevention or reduction of gall stone formation. As body fat composition may be correlated with certain types of cancers, the present compositions may be useful for the prevention or amelioration of certain types of cancers. The present invention also includes methods for manufacture of a medicament for use in conjunction with the cosmetic/therapeutic conditions described herein, containing at least one of the present compositions.

The present compositions and methods may be used in conjunction with other medicaments, such as those useful for the treatment of diabetes (e.g., insulin or analogs thereof, thiazolidinediones or other antihyperglycemic agents, and possibly amylin or antagonists there of), cholesterol and blood pressure lowering medicaments (such as those which reduce blood lipid levels or other cardiovascular medicaments), and activity increasing medicaments (e.g., amphetamines). Appetite suppressants may also be used (such as serotonin modulators and neuropeptide Y antagonists).

Such administration may be simultaneous or may be in seriatim.

In addition, the present methods may be used in conjunction with surgical procedures, such as cosmetic surgeries designed to alter the overall appearance of a body (e.g., liposuction or laser surgeries designed to reduce body mass, or implant surgeries designed to increase the appearance of body mass). The health benefits of cardiac surgeries, such as bypass surgeries or other surgeries designed to relieve a deleterious condition caused by blockage of blood vessels by fatty deposits, such as arterial plaque, may be increased with concomitant use of the present compositions and methods. Methods to eliminate gall stones, such as ultrasonic or laser methods, may also be used either prior to, during or after a course of the present therapeutic methods. Furthermore, the present methods may be used as an adjunct to surgeries or therapies for broken bones, damaged muscle, or other therapies which would be improved by an increase in lean tissue mass.

In yet another aspect, the present invention provides for methods of manufacture of a medicament for the treatment of obesity, type II diabetes, excess blood lipid, or cholesterol levels, increasing sensitivity to insulin, increasing lean mass, and other conditions as set forth above. Also provided are solely cosmetic treatments for individuals wishing to improve appearance by weight loss, and more specifically, loss of fat deposits, even in the absence of any therapeutic benefit.

Diagnostic Compositions and Methods

As indicated supra, polypeptide products of the invention may be "labeled" by association with a detectable marker substance (e.g., radiolabeled with

^{125}I, fluorescent, chemiluminescent, enzyme) to provide reagents useful in detection and quantification of OB receptor (or complexes) in solid tissue and fluid samples such as blood or urine. Nucleic acid products of the invention may also be labeled with detectable markers (such as radiolabels and non-isotopic labels such as biotin) and employed in hybridization processes to locate the human OB receptor gene position and/or the position of any related gene family in a chromosomal map. Nucleic acid sequences which selectively bind the human OB receptor gene are useful for this purpose. They may also be used for identifying human OB receptor gene disorders at the DNA level and used as gene markers for identifying neighboring genes and their disorders. Such nucleic acid sequences may be sued for detection or measurement of OB receptor mRNA level from a biological sample. Contemplated herein are kits containing such labelled materials.

The protein and/or nucleic acids provided herein may also be embodied as part of a kit or article of manufacture. Contemplated is an article of manufacture comprising a packaging material and one or more preparations of the presently provided compositions. Such packaging material will comprise a label indicating that the protein or nucleic acid preparation is useful for detecting and/or quantifying the amount of OB receptor in a biological sample, or OB receptor defects in a biological sample. As such, the kit may optionally include materials to carry out such testing, such as reagents useful for performing DNA or RNA hybridization analysis, or PCR analysis on blood, urine, or tissue samples.

A further embodiment of the invention is selective binding molecules, such as monoclonal antibodies selectively binding OB receptor. The

hybridoma technique described originally by Kohler and Milstein Eur. J. Immunol. 6, 511-519 (1976) has been widely applied to produce hybrid cell lines that secrete high levels of monoclonal antibodies against many specific antigens. Recombinant antibodies, (see Huse et al., Science 246: 1275 (1989)) may also be prepared. Such recombinant antibodies may be further modified, such as by modification of complementarity determining regions to increase or alter affinity, or "humanizing" such antibodies. Such antibodies may be incorporated into a kit for diagnostic purposes, for example. A diagnostic kit may be employed to determine the location and/or amount or OB receptor of an individual. Diagnostic kits may also be used to determine if an individual has receptors which bind OB protein, or those which, to varying degrees, have reduced binding capacity or ability. As stated infra, such antibodies may be prepared using immunogenic portions of an OB receptor protein. Such selective binding molecules may themselves be alternatives to OB protein, and may be formulated for pharmaceutical composition.

Such proteins and/or nucleic acids may be used for tissue distribution assays (for example, as provided in the working example below) or for other assays to determine the location of OB receptor.

The present OB receptor protein family may be used in methods to obtain OB protein analogs, mimetics or small molecules. One would simply prepare a desired OB receptor protein, particularly one with capability of binding to native OB protein, and assay the test molecule, which may be labelled with a detectable label substance, for ability to bind to such receptor. Other parameters, such as affinity, and location of binding, may also be ascertained by methods available to those skilled in the art. For example, one could use portions of the present OB receptors, particularly portions in

the extracellular domain which are necessary for ligand binding, to determine the location of such binding. One could prepare OB receptors which have various truncations or deletions of regions of the extracellular domain which could be used to determine the location of test molecule binding. One could use an OB receptor known to be defective in native OB binding, such as potentially one from an individual having such defective receptors, and use this as the basis for ascertaining OB protein which would be effective to result in desired biological activity (i.e., weight loss, reduction in blood dyslipidemias or lowering of cholesterol levels, reduction in incidence or severity of diabetes). Other uses include solely cosmetic uses for alteration of body appearance, particularly the removal of fat.

The present OB receptor protein or nucleic acids may also be useful to identify substances which "up-regulate" OB protein or receptor. For instance, the temporal expression of OB receptor in vivo may be useful to determine if an administered substance causes an increase or decrease in OB receptor. One may conclude that an increase in OB receptor expression results in modultion of weight or lipid metabolism.

The divergence in the C-terminus may represent OB receptors with different signal transduction abilities. Therefore the different receptor family members may be used for different assays, depending on the type of signal transduction observed. It is thought that at least a portion of the intracellular domain is necessary for signal transduction (see supra).

The following examples are offered to more fully illustrate the invention, but are not to be construed as limiting the scope thereof.

EXAMPLE 1: IDENTIFICATION OF HUMAN OB RECEPTOR PROTEIN

Human OB receptor protein DNA was identified in a human liver cDNA library in two steps. The first step used two primers in polymerase chain reaction (PCR) to amplify a selected 300 base pair region from the human liver cDNA library. The second step used the PCR fragment as a probe to screen the human liver cDNA library. Thirteen clones were obtained, but these were incomplete at the 5' end. A procedure was performed to complete the 5' end to make complete clones. Twelve clones were sequenced. These twelve clones were identified as either "A", "B" or "C" as denoted by the C-terminus of the predicted amino acid sequence.

Polymerase Chain Reaction.

The original PCR primer was based on the 5' end and the 3' end of a 416 base pair sequence having GenBank Database Accession No. T73849. This sequence was selected on the basis of a known motif present in cytokine receptors, "WSXWS".

The 5' primer had the sequence 73-96 of the 416 bp sequence. The 3' primer had the sequence 337-360 of the 416 bp sequence.

These primers were used to probe a human cDNA liver library (Stratagene). Standard methods were used.

This resulted in a PCR fragment having the sequence 73-360 of the 416 bp fragment.

Hybridization.

The 300 bp PCR fragment was used to probe a human liver cDNA library (Stratagene) using standard methods. This second hybridization resulted in 13 positive clones. These were partial clones, incomplete at the 5' end.

Completion of the 5' end.

Rapid Amplification of cDNA End ("RACE", kit, GIBCO/BRL) was used to obtain the full length clones.

Sequencing results.

Sequencing revealed the three types of OB receptor DNAs. Of the thirteen clones, 4 clones were the "A" type (Seq. ID Nos. 1 and 2); 1 clone was the "B" type (Seq. ID Nos. 3 and 4) and 4 clones were of the "C" type (Seq. ID Nos. 5 and 6).

As can be seen from the Sequence Identifications (below), OB receptor A is 896 amino acids long, "B" is 904 amino acids long, and "C" is 958 amino acids long. These different OB receptors are identical at amino acid positions 1-891, and diverge almost completely beginning at position 892. The leader sequence is postulated to be, by hydrophobicity analysis, amino acids 1-21(M-A), 1-22(M-F) or 1-28(M-I), with the mature protein beginning at positions 22(F), 23(N) or 29(T). Based on hydrophobicity analysis, the leader sequence is most likely to be at positions 1-21(M through A). Chinese Hamster Ovary Cell ("CHO") cell production of the secreted form of OB receptor protein also produced a protein having amino acid number 22 as the first amino acid of the mature protein. The transmembrane region is likely to begin at either position 840 (A) or 842(L) through position 862(I), 863(S) or 864(H). For OB receptor type "A", the last amino acid is located at position 896 and is a lysine (L). For OB receptor type "B", the last amino acid is located at position 904 and is a glutamine (Q). For OB receptor type "C", the last amino acid is located at position 958 and is glutamic acid (E).

For OB receptor protein type "C", the C-terminal region possesses high homology to a known human

transposable element. From nucleotide 2737 through 2947 of the present human OB receptor protein type "C", there is a 98.1% homology with a 211 base section of a human retrotransposable element described in Ono et al., Nucl. Acids Res. 15: 8725-8737 (1987) (bases 520 through 731, SINE-R11, GENBANK accession no. x07417).

EXAMPLE 2: TISSUE DISTRIBUTION

Tissue distribution was ascertained using two methods. The first method involved using the entire type "A" OB receptor. The second method involved using probes which are specific to the C-terminal region of the protein. Since these C terminal regions are divergent, the second method detected the tissue distribution of the different members of the OB receptor family.

The first method used a Northern Blot kit (Clontech), using the entire type A OB receptor DNA as a probe. The second method used PCR with primers specific to the nucleic acids encoding the divergent C terminus of the three types. Standard methods were used.

Table 2 shows the results for the Northern Blot and the PCR methods. The "+" indicates the investigator's subjective determination of the strength of signal. For the Northern Blot analysis, a triple "+++" indicates that a result (a dark "band" on the X-ray film) was seen upon overnight exposure of the film. A double "++" indicates that bands were seen at two weeks of exposure. A single "+" indicates that the bands were seen after three weeks of exposure. In addition, using this method, two molecular weights were observed, one at 4 Kb and one at 6.2 Kb. Although distribution was ubiquitous, the strongest signals were seen for ovary, heart and liver. For the PCR analysis, OB receptor "A" was seen in all tissue types tested (prostate, ovary, small intestine, heart, lung, liver

and skeletal muscle), type "B" was seen only in lung and liver, and type "C" was seen in ovary, heart, lung and liver.

Table 2

Tissue Distribution of the Novel OB Receptor

	Northern Blot			PCR		
	4 Kb	6.2 Kb		A	B	C
Spleen	-	+				
Thymus	-	+				
Prostate	-	+		+	-	-
Testis	-	+				
Ovary	-	+++		+	-	+
Small Intestine	-	++		+	-	-
Colon	-	-				
Peripheral blood Leukocyte	-	-				
Heart	-	+++		+	-	+
Brain	-	-				
Placenta	-	+				
Lung	+	++		+	+	+
Liver	+++	+++		+	+	+
Skeletal Muscle	-	++		+	-	-
Kidney	-	++				
Pancreas	-	+				

EXAMPLE 3: IDENTIFICATION OF HUMAN OB RECEPTOR GENOMIC DNA AND CHROMOSOME LOCALIZATION; IDENTIFICATION OF HUMAN OB RECEPTOR "D"

The full length human OB receptor genomic DNA was also prepared. OB receptor "A" cDNA, in its entirety, was used as a probe against a human genomic DNA library, using materials and methods from a commercially available kit (Genome Systems, using a human genomic library in a P1 vector). A single

positive clone was detected. There are introns located at (with respect to OB receptor "A" DNA) base pair number: 559, 1059, 1350, 1667, 1817, 1937, 2060, 2277, 2460, 2662, and 2738.

The human OB receptor gene was localized to human chromosome 1P31 by FISH analysis (Genome Systems). Human chromosome 1 is thought to correspond to mouse chromosome 4C7, which is presumed to be the location of the *db* locus.

A further chromosomal sequence was isolated. This chromosomal DNA sequence was isolated from a human genomic library as described above. This chromosomal sequence encodes what is here denominated human OB receptor "D", and the encoded amino acid sequence is set forth in SEQ. ID No. 7. A cDNA encoding this amino acid sequence is set forth in SEQ. ID No. 8. The chromosomal DNA intron/exon junction map is set forth as SEQ. ID No. 9.

As with forms "A", "B", and "C", for the present form "D" OB receptor protein, the first amino acid of the mature protein is likely (using hydrophobicity analysis) to begin at position 22 (F), 23 (N) or 29 (T). The last amino acid of the protein is at position 1165 and is a valine residue. As with the other forms, the extracellular domain extends from position 22 (F), 23 (N) or 29 (T) to position 839 (D) or 841 (G). The transmembrane domain appears to begin at position 840 (A) or 842 (L). The end of the transmembrane domain appears to be located at position 862 (I), 863 (S) or 864 (H). The C-terminal region, beyond the transmembrane region, is likely to be involved in signal transduction, and is located at position 863 (S), 864 (H) or 865 (Q) through position 1165 (V).

The present OB receptor form "D" is identical to that published by Tartaglia et al, Cell 83: 1263-1271

(December 29, 1995) with the exception of a single amino acid change at amino acid position 976 (nucleotide codon begining at position 3022). The present type "D" amino acid at position 976 is aspartic acid, and the published amino acid corresponding to the same position is alanine. This is a non-conservative substitution, see infra, and since the location of the substitution is within a region thought important for signal transduction, this change could affect the function of the molecule.

EXAMPLE 4: PREPARATION OF SOLUBLE OB RECEPTOR

Three forms of soluble human OB receptor have been prepared:

1. Leader + Extracellular Domain (Seq. ID Nos. 10 and 11): A recombinant form of the soluble human OB receptor was prepared. This form encompasses, in the immature protein, the leader sequence and the extracellular domain (amino acids 1-839). The mature protein would have the leader sequence deleted, and the first amino acid of the mature recombinant soluble human OB receptor would be 22 (F), 23 (N) or 29 (T). This protein was expressed as described below.

2. Leader + Extracellular Domain + C-terminal FLAG (Seq. ID No. 12): A second form of the recombinant soluble human OB receptor was also prepared. This form had a "FLAG" tag located at the "C" terminus of the protein. The "FLAG" peptide is a useful research tool as it allows one to follow the protein using an antibody which recognizes the "FLAG" peptide. Such reagents are commercially available (IBI, New Haven, CT). This protein was expressed as described below.

3. Native Splice Variant (Seq. ID Nos. 13 and 14): This form is believed to the the recombinant form of a naturally occurring secreted,

soluble human OB receptor. This form has most of the amino acids found in the extracellular domain (amino acids 22-798), and a unique 6 amino acid sequence at the carboxyl terminus. Beginning at amino acid position 799 of Seq. ID No. 13, the amino acid sequence of this native splice variant human OB receptor protein is "G K F T I L."

EXAMPLE 5: PREPARATION OF EXPRESSION VECTORS

Recombinant human OB receptor expression vectors have been prepared for expression in mammalian cells. As indicated above, expression may also be in non-mammalian cells, such as bacterial cells. The type "A" cDNA (Seq. ID No. 2) was placed into a commercially available mammalian vector (pCEP4, Invitrogen) for expression in mammalian cells, including the commercially available human embryonic kidney cell line, "293".

Recombinant human OB receptor expression vectors have been prepared for expression of recombinant soluble OB receptor, consisting of the leader sequence and the extracellular domain (Seq. ID Nos. 10 and 11), using the same system as above (the commercialy available mammalian vector pCEP4, and "293" cells). This recombinant soluble human OB receptor was also expressed in CHO cells in a similar way.

The "FLAG-tagged" form (Seq. ID No. 12) of the recombinant soluble human OB receptor, and the "D" form (Seq. ID No. 7) were also expressed in "293" cells in a similar fashion as above.

Detection of desired protein was accomplished using BIACORE (Pharmacia) analysis. This analysis is analogous to that described in Bartley et al., Nature 368: 558-560 (1994).

Essentially, the BIACORE machine measures affinity interactions between two proteins. In this

case, the OB protein was immobilized on the machine, and conditioned media from cell lines expressing the OB receptor was added to the machine. Any receptor protein present in the conditioned media bound to the OB protein surface. The BIACORE machine gave a read-out indicating that receptor protein was being expressed. For recombinant soluble receptor (Seq. ID No. 10) expression in "293" cells, the read-out was 191.0 relative to a baseline readout of 0. For recombinant soluble receptor (SEq. ID No. 10) expression in CHO cells, the read-out was 150.9 relative to a baseline readout of 0. For recombinant soluble receptor with a C-terminal FLAG-tag (Seq. ID. No. 12), the read-out was 172.0 relative to a baseline of 0.

For expression in bacterial cells, one would typically eliminate that portion encoding the leader sequence (e.g., potentially amino acids 1-21, 1-22 or 1-28). One may add an additional methionyl at the N-terminus for bacterial expression. Additionally, one may substitute the native leader sequence with a different leader sequence, or other sequence for cleavage for ease of expression.

EXAMPLE 6: DEMONSTRATION OF SIGNAL TRANSDUCTION

This example demonstrates that the "D" form is active to produce a signal within a cell, whereas in the same cell type, the "A" form does not. The signal transduction assay was performed by the use of "293" cells transiently expressing either the "A" or the "D" form (see above for preparation of the "293" expression clones). Phosphorylation of molecules predicted to be involved in signal transduction within the cell was examined upon OB protein binding to the OB receptor protein tested. The results demonstrate that upon binding of OB protein to the extracellular domain, the

"D" form of the present OB protein receptor transduces a signal sufficient to initiate phosphorylation of signalling molecules.

Methods

1. OB receptor molecules. As indicated above, the "A" form (Seq. ID No. 1) and the "D" form (Seq. ID. No. 7) were studied.

2. Expression system. The pCEP 4 system (as described above) having inserted DNA encoding the "A" form (Seq. ID No. 2) or the "D" form (Seq. ID No. 8) was used to transfect "293" cells. These cells did not allow for the pCEP4 vector to integrate into the genome, so such expression was transient. Non-recombinant (mock-transfected) cells were also prepared as controls.

3. Detection of phosphorylation. Mock transfected cells and cells expressing the "A" form or the "D" form were analyzed. Prior to treatment the cells were serum-starved by incubation in media with 0.5% serum for 16 hours prior to the treatments. The cells were treated with the OB protein (10 mg/ml) for 15 minutes at 37°C, after which the cells were lysed in modified NP40 buffer (50 mM Tris, pH 8.0, 150 mM sodium chloride, 1% NP40, 10 mg/ml aprotinin, 5mM EDTA, 200 mM sodium orthovanadate). Phosphotyrosine containing proteins were immunoprecipitated (Anti-phosphotyrosine antibody 4G10, UBI, Lake Placid, NY), and separated by SDS polyacrylamide gel electrophoresis. After electrophoresis and electroblotting to membranes the immunoprecipitates were probed with antibodies to various signal transduction molecules. Antibodies to STATs, JAKs and ERKs were purchased from Santa Cruz Biotechnology Inc. Immune complexes were detected by horseradish-peroxidase conjugated secondary reagents using chemiluminescence as described by the manufacturer (ECL, Amersham). As a positive control, 32D cells were

treated with IL-3, which is known to activate by tyrosine phosphorylation most of the molecules being analyzed.

4. Results. Results are presented in Table 3, below. As can be seen, only the "D" form was able to respond to either mouse or human OB protein as detected by phosphorylation of JAK and STAT molecules. A "+" designation indicates signal was detected, a "-" designation means that no signal was observed.

TABLE 3

Signal /AB‡	293 Alone	293/D hrOB*	293/D mrOB**	293/A hrOB#	293/A mrOB##	32D IL-3
STAT1	-	+				
STAT3	-	+	+	-	-	+
STAT5	-	+	+			+
JAK1	-	+	+	-	-	+
JAK2	-	+	+	-	-	+
JAK3	-	-	-			-
TYK2	-	+	+			-
ERKs 1,2	-	-	-	-	-	+

‡ Antibody detection target

* 293 cells expressing receptor form "D", treated with recombinant human OB

** 293 cells expressing receptor form "D" treated with recombinant murine OB

293 cells expressing receptor form "A" treated with recombinant human OB

293 cells expressing receptor form "A" treated with recombinant murine OB

The "D" form is capable of initiating signalling through the JAK/STAT pathways in 293 cells, whereas the "A" form cannot.

EXAMPLE 7: USE OF SOLUBLE OB RECEPTOR AS A THERAPEUTIC

This example demonstrates that soluble OB receptor protein acts to protect the activity of OB protein. Below, soluble OB receptor and/or OB protein was delivered to a mammal via "gene transplant" -- that is, via bone marrow cells engineered to express the desired DNAs. When soluble OB receptor combined with OB protein was delivered, the animals lost more weight than delivery of OB protein alone. This demonstrates the protective activity of OB receptor protein.

While not wishing to be bound by theory, one explanation of the mode of action is that soluble OB receptor protein acts to protect the OB protein in serum from agents or conditions which could diminish its activity. The protective action appears to increase circulating half-life of the protein. As such, the present example demonstrates that OB receptor either alone, or administered as a complex with OB protein (or analog or derivative thereof) could act as a therapeutic agent.

Materials and methods:

1. Preparation of recombinant *ob* retroviral vector Packaging Cells.

Use of murine ob cDNA. Full length wild-type murine *ob* cDNA was amplified by the PCR using synthetic oligonucleotides designed from the published sequence Zhang et al., Nature 372: 425-432 (1994).Linkers (An Eco RI linker and a Bgl II linker) were used to facilitate subcloning.

Use of soluble recombinant human OB receptor cDNA. Methods similar to those above were used. A construct containing the recombinant human soluble receptor of Seq. ID No. 10 was used, and modified with

linkers to facilitate cloning (i.e., the addition of a Bgl II restriction endonuclease recognition site).

Placement of desired cDNA into vector. PCR products were digested with *Eco*RI and *Bgl*II and cloned into similarly-digested parental vector (pMSCV2.1) under the transcriptional control of the viral LTR promoter. The parental MSCV vector (supplied by R. Hawley, University of Toronto, Canada) was derived from MESV (murine embryonic stem cell virus) and contains a neomycin phosphotransferase resistance (neo^r) gene driven by an internal mouse phosphoglycerate kinase (PGK) promoter, as described· Hawley, et al, J. Exp. Med. 176: 1149 -1163 (1992). The parental plasmid pMSCV2.1 and pMSCV-OB were independently electroporated into the GP+E-86 packaging cell line (supplied by Dr. A. Bank, Columbia University, NY) Markowitz et al., J. Virol. 62:1120-1124 (1988). Transient supernatants were harvested from electroporated populations and used to infect tunicamycin treated parental GP+E-86 cells. Tunicamycin treatment relieves the block to superinfection of the parental packaging cells. G418 (0.78 mg/mL, 67% active, GIBCO Laboratories, Life Technologies, Inc., Grand Island, NY) resistant clones were selected from each infected population and titered by infection of NIH3T3 cells. Clones with the highest G418 resistant titer were expanded and frozen as aliquots. Each bone marrow infection and transplantation experiment used aliquots from the same passage of frozen viral packaging cells. Both the parental and *ob* packaging cell lines were tested for the presence of, and found to be free from, replication competent virus using a sensitive marker rescue assay. Moore, et al., (1993) in: Gene Targeting: A Practical Approach, Joyner, Ed. (Oxford University Press, New York, NY).

2. Production of Retroviral Supernatants.

Recombinant virus-producing packaging cell lines were grown in 175cm^2 tissue culture flasks in Iscove's Modified Dulbecco's Medium (IMDM) (GIBCO), 10% (v/v) FBS, at 37°C. Sub-confluent (approximately 60%) monolayers of cells were fed with fresh medium 24h prior to harvest of virus-containing supernatants. Viral supernatants were removed from packaging cell lines by aspiration, sterile filtered (0.45mM) and added directly to bone marrow cultures. Fresh aliquots of frozen packaging cell lines were thawed for use in each experiment.

3. Bone Marrow Infection and Transplantation.

Eight to 12-week old female C57BL/6J (+/+) or (*ob/ob*) mice were used as bone marrow donors and recipients. All mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and housed under specific pathogen-free conditions in a vivarium in accordance with governmental regulations and institutional guidelines.

Bone marrow cells were harvested from femurs and tibias of donor mice 4 days post 5-fluorouracil (5-FU, Sigma Chemical Co., St. Louis, MO) treatment (150 mg/kg, i.v.). Bone marrow cells (6 X 10^5/mL) were incubated in 150mm tissue culture dishes (30mL/dish) containing fresh viral supernatant (as described above), 15% FBS, 6 mg/mL polybrene (Sigma), 0.1% bovine serum albumin (BSA, Fraction V, Sigma), 2.5 ng/mL recombinant mouse IL-3 (rmIL-3), 100 ng/mL each of recombinant human IL-6 (rhIL-6), recombinant human IL-11 (rhIL-11), and recombinant rat SCF (rrSCF). All growth factors were produced by Amgen, Inc. (Thousand Oaks, CA). Culture media were replaced daily for 3 days with fresh virus-containing supernatant and growth factors.

At the end of the infection period, total non-adherent and adherent cells were washed and resuspended in 1% BSA-saline and transplanted into g-irradiated (12

Gy, Cs-137) mice. Each animal was transplanted with 2.5 X 10^6 syngeneic cells. There were approximately 10 animals per cohort.

4. Analysis of OB protein expression in transfected cells and transplanted animals. For transfected bone marrow cells, Western analysis was performed. Vector packaging cell supernatant was resolved by SDS-PAGE (16% acrylamide), then transferred to Hybond-ECL (Amersham, Arlington Heights, IL). The filter was incubated with affinity-purified rabbit a-mouse OB protein polyclonal antibody (1mg/mL) in T-TBS buffer (20mM Tris-chloride, pH7.6, 137mM NaCl, 0.1% Tween20) at room temperature for 45 min. Horseradish peroxidase (HRP)-conjugated donkey a-rabbit IgG (Amersham) was diluted in T-TBS (1:2500) and incubated with the filter at room temperature for 45 min. Enhanced chemiluminescence (ECL, Amersham) detection was performed as recommended by the manufacturer.

For transplanted animals, serum was analyzed. Animals were bled retroorbitally, under isofluorane anesthesia. Serum from transplanted *ob/ob* animals was resolved by SDS-PAGE (4-20% acrylamide) under non-reducing and reducing conditions, then transferred to Trans-Blot (Bio-Rad Laboratories, Hercules, CA) membranes. The membranes were incubated for 2 hours at room temperature with HRP-conjugated rabbit a-mouse OB protein antibody (0.125mg/mL) in T-TBS buffer containing 5% fetal bovine serum and 1% bovine serum albumin. Bound OB protein was detected by ECL (Amersham), performed as recommended by the manufacturer.

For quantitation of soluble OB protein levels, serum from transplanted animals was subjected to ELISA analysis. Briefly, affinity-purified rabbit a-OB protein polyclonal antibody was coated onto 96-well plates. Standards (purified recombinant OB protein

monomer, Pelleymounter et al., Science 269: 540-543 (1995) and experimental samples were added, and the plates were incubated at room temperature. The plates were washed twice and affinity-purified rabbit a-OB protein antibody conjugated to horseradish peroxidase was added. Following incubation at room temperature, the plates were washed four times with TNE-Tween20. TMB/peroxide substrate was added and the color reaction was read at 450nm in a Molecular Devices plate reader. OB protein concentrations in sera were estimated by comparison to a standard curve prepared from internal standards. OB protein levels were reliably measured in samples containing >160 pg/mL.

5. Body Weight and Food Intake. Mice were offered pelletized rodent chow (PMI Feeds, Inc., St. Louis, MO) ad libitum. The body weight of individual animals was measured daily for the first two months of analysis, and weekly thereafter. Food consumption was measured daily on selected groups of individually-housed animals.

Results

Results are presented in Tables 4 and 5 below. Administration of OB protein receptor increased the effectiveness of OB protein. This may have been accomplished via an increased circulation time of OB protein in the presence of OB protein receptor.

As can be seen in the Table, animals administered a combination of OB protein and OB protein receptor (via genetic therapy) had a greater weight loss after 28 days than either composition alone. The Table presents the results of two experiments ("___/___"). As can be seen, use of the OB protein alone at day 40 resulted in animals with 87.5% and 72.2% of the starting weight. Using OB receptor in combination with OB protein, however, resulted in animals with 68% and

53.6% of the starting weight. Use of the receptor alone appeared to have little effect, if any.

TABLE 4

Treatment	Weight (g) decrease at day 28 (ave)	% starting weight (ave) day 28	% starting weight (ave) day 40
OB alone*	6.3/12.7	87.9/75.3	87.5/72.2
Receptor** alone	[1.4]/[0.3]	103/100.6	104.2/101.7
OB + Receptor***	12.6/16.8	76.3/67.5	68/53.6

* 50% bone marrow cells transfected with OB protein cDNA as described above, and 50% bone marrow cells without genetic alteration

** 50% bone marrow cells transfected with OB receptor protein cDNA as described above, and 50% bone marrow cells without genetic alteration

*** 50% bone marrow cells transfected with OB protein cDNA as described above, and 50% bone marrow cells transfected with OB receptor protein cDNA as described above.

Table 5, below, contains results of the OB levels found in the serum from animals administered OB protein alone, or administered OB protein in combination with OB protein receptor (via the "gene therapy" method of this example). The data reflect nanograms of OB protein per milliliter of serum, plus or minus the standard error of the mean.

TABLE 5

Treatment	Experiment #1‡	Experiment #2‡‡
OB alone*	2.93 +/- 0.77	9.74 +/- 1.02
Receptor** alone	0.08 +/- 0.05	0.12 +/- 0.07
OB + Receptor***	12.11 +/- 1.90	15.18 +/- 2.52

* 50% bone marrow cells transfected with OB protein cDNA as described above, and 50% bone marrow cells without genetic alteration

** 50% bone marrow cells transfected with OB receptor protein cDNA as described above, and 50% bone marrow cells without genetic alteration

*** 50% bone marrow cells transfected with OB protein cDNA as described above, and 50% bone marrow cells transfected with OB receptor protein cDNA as described above.

‡ Experiment #1 was conducted as described above, with OB protein serum levels measured after 38 days.

‡‡ Experiment #2 was also conducted as described above, with OB protein serum levels measured after 24 days.

The data demonstrate the protective effects of OB receptor. As can be seen, in the presence of OB receptor, OB protein has a higher accumulation in the serum. The degree of accumulation is observed to increase inversely with the levels of OB protein in the serum. In Experiment #1 (with a base OB protein level of about 2.93 ng/ml), the OB protein serum level increased about 400% with the addition of receptor, where in Experiment #2 (with a base of about 9.74), the OB protein serum level increased by about 25%.

OB receptor administered either alone or in association with OB protein (or analogs or derivatives

thereof) may serve to increase the circulation time of OB protein, and therefore enhance the therapeutic efficacy of either exogenous or endogenous OB protein.

EXAMPLE 8: PREPARATION OF SELECTIVE BINDING MOLECULES

Animals were immunized for the preparation of polyclonal antibodies using the following peptides (with respect to the numbering of the amino acids for OB receptor A, Seq. ID No. 1): 54-64; 91-100; 310-325; 397-406; 482-496; 874-885; and, with respect to amino acids of OB receptor "C" (Seq. ID No. 5), 910-929. Some of the polyclonal antibodies prepared (in rabbits) were tested for ability to bind to recombinant human OB receptor protein. The polyclonal antibody prepared against amino acids 54-64 was found to have the highest affinity for recombinant human OB receptor protein. The polyclonal antibody prepared against amino acids 397-406 was also found to bind to recombinant human OB receptor protein. The polyclonal antibody prepared against amino acids 91-100 was found to slightly bind to recombinant human OB receptor protein. The polyclonal antibody prepared against amino acids 874-885 was found not to bind to recombinant human OB receptor protein.

An additional study was performed which demonstrates the expression and purification of the extracellular domain of the OB receptor protein in CHO cells, and antibodies which recognize this OB protein receptor extracellular domain.

The extracellular domain of the human OB receptor protein was expressed as a secreted, soluble protein in CHO cells as previously described supra. Individual cell lines were isolated and grown in increasing amounts of methotrexate to increase selection/expression of the recombinant receptor protein (100, 200 or 500 micrograms methotrexate per ml of media). Conditioned media from the CHO cell lines was

collected, and the proteins in the conditioned media were fractionated by SDS-PAGE. The OB receptor extracellular domain migrated as a broad band with an apparent size range of about 140 kDa to about 200 kDa. The OB receptor protein extracellular domain was detected by Western Blot analysis using polyclonal antibodies prepared against a portion of the extracellular domain of the OB receptor protein. The unfolded, bacterially expressed protein was used as an antigen to generate antisera in rabbits. The identified OB receptor extracellular domain was purified by affinity chromatography. The purified protein was sequenced at the amino terminus to confirm that it was the OB receptor and also to determine the start of the mature protein (after signal peptide cleavage) as expresed in CHO cells. It was found that amino acid no. 22 (according to the amino acid sequence numbering of Seq. ID No. 1, infra), was the first amino acid of the mature protein as expressed in CHO cells.

Other immunogenic peptides may be used. Polyclonal, monospecific polyclonal, monoclonal, antibody fragments, and recombinant antibodies may be prepared using methods available to those skilled in the art.

One may further use recombinant techniques or peptide synthesis methods to alter the character of such selective binding molecules. This may be accomplished by preparing recombinant antibodies having altered complementarity determining regions (sometimes referred to in the art as "CDR's") to, for example "humanize" the antibodies by using human F_C (constant) regions. Other types of recombinant antibodies, for example, those having CDR's altered to enhance affinity or selectivity to one or more members of the OB receptor family, may be prepared and used using methods available to those

skilled in the art. See Winter et al., Nature 349: 293-299 (1991).

The present OB receptor protein may be used as an assay to screen for desired selective binding molecules. Such assay may be based on binding capability, or biological activity, or, other means of detecting signal transduction. For example, if one were to prepare a series of modified antibodies, one could test them for affinity (i.e, binding strength) against the target OB receptor.

The selective binding molecules may be useful for diagnostic purposes, such as tissue distribution analysis, or to diagnose the relative affinity of an individual's OB receptors for such selective binding molecule to determine the functionality of an individual's OB receptor during a course of therapy. Selective binding molecules may be alternative therapeutic or cosmetic products to OB protein.

EXAMPLE 9: GENE THERAPY

One may deliver the present OB receptor protein via gene therapy, as described infra.

One may envision, using materials and methods available to those skilled in the art and provided herein, using T-cells as an agent carrying DNA expressing OB receptor for gene therapy. An individual would have T-cells selected using CD34+ selection and a magnetic microparticles selection device. Such cells would be transfected with the desired DNA, or the regulation of the desired coding region may be altered using homologous recombination or other in situ techniques. The transduced cells could be selected empirically, using means to detect the desired protein, or a marker may be included which permits indirect detection (i.e., a selectable marker as is known in the

art). Optionally, such cells could be expanded, for example, using one or more growth factors such as SCF or an interleukin, and such cells could be stored for future use. In such a way, the procedure would only have to be accomplished once or infrequently in an individual's lifetime, for later transfer into the individual. The cells would be re-planted into the individual, and the individual would be monitored for desired therapeutic effect, such as weight loss/maintenance of weight, diabetes recurrence, blood lipid levels, or other conditions.

Illustrative Nucleic Acid and Amino Acid Sequences

The below amino acid and DNA sequences are those to which reference has been made. An asterick("*") indicates the position of a stop codon.

Human OB Receptor "A" Amino Acid Sequence (Seq. ID No. 1 (Amino Acid, single letter abbreviation):

```
1    MICQKFCVVL LHWEFIYVIT AFNLSYPITP WRFKLSCMPP NSTYDYFLLP
51   AGLSKNTSNS NGHYETAVEP KFNSSGTHFS NLSKTTFHCC FRSEQDRNCS
101  LCADNIEGKT FVSTVNSLVF QQIDANWNIQ CWLKGDLKLF ICYVESLFKN
151  LFRNYNYKVH LLYVLPEVLE DSPLVPQKGS FQMVHCNCSV HECCECLVPV
201  PTAKLNDTLL MCLKITSGGV IFQSPLMSVQ PINMVKPDPP LGLHMEITDD
251  GNLKISWSSP PLVPFPLQYQ VKYSENSTTV IREADKIVSA TSLLVDSILP
301  GSSYEVQVRG KRLDGPGIWS DWSTPRVFTT QDVIYFPPKI LTSVGSNVSF
351  HCIYKKENKI VPSKEIVWWM NLAEKIPQSQ YDVVSDHVSK VTFFNLNETK
401  PRGKFTYDAV YCCNEHECHH RYAELYVIDV NINISCETDG YLTKMTCRWS
451  TSTIQSLAES TLQLRYHRSS LYCSDIPSIH PISEPKDCYL QSDGFYECIF
501  QPIFLLSGYT MWIRINHSLG SLDSPPTCVL PDSVVKPLPP SSVKAEITIN
551  IGLLKISWEK PVFPENNLQF QIRYGLSGKE VQWKMYEVYD AKSKSVSLPV
601  PDLCAVYAVQ VRCKRLDGLG YWSNWSNPAY TVVMDIKVPM RGPEFWRIIN
651  GDTMKKEKNV TLLWKPLMKN DSLCSVQRYV INHHTSCNGT WSEDVGNHTK
701  FTFLWTEQAH TVTVLAINSI GASVANFNLT FSWPMSKVNI VQSLSAYPLN
751  SSCVIVSWIL SPSDYKLMYF IIEWKNLNED GEIKWLRISS SVKKYYIHDH
801  FIPIEKYQFS LYPIFMEGVG KPKIINSFTQ DDIEKHQSDA GLYVIVPVII
851  SSSILLLGTL LISHQRMKKL FWEDVPNPKN CSWAQGLNFQ KRTDIL*SLI
901  MITTDEPNVP TSQQSIEY*K IFTF*RRGAN LKKIQLNF*E LTYGGLC*FR
951  T*NRCVNLGS KCRFESSLDV *L
```

Human OB Receptor "A" DNA Sequence (Seq. ID No. 2 (DNA)):

```
1     CCGCCGCCAT CTCTGCCTTC GGTCGAGTTG GACCCCCGGA TCAAGGTGTA
51    CTTCTCTGAA GTAAGATGAT TTGTCAAAAA TTCTGTGTGG TTTTGTTACA
101   TTGGGAATTT ATTTATGTGA TAACTGCGTT TAACTTGTCA TATCCAATTA
151   CTCCTTGGAG ATTTAAGTTG TCTTGCATGC CACCAAATTC AACCTATGAC
201   TACTTCCTTT TGCCTGCTGG ACTCTCAAAG AATACTTCAA ATTCGAATGG
251   ACATTATGAG ACAGCTGTTG AACCTAAGTT TAATTCAAGT GGTACTCACT
301   TTTCTAACTT ATCCAAAACA ACTTTCCACT GTTGCTTTCG GAGTGAGCAA
351   GATAGAAACT GCTCCTTATG TGCAGACAAC ATTGAAGGAA AGACATTTGT
401   TTCAACAGTA AATTCTTTAG TTTTTCAACA AATAGATGCA AACTGGAACA
451   TACAGTGCTG GCTAAAAGGA GACTTAAAAT TATTCATCTG TTATGTGGAG
501   TCATTATTTA AGAATCTATT CAGGAATTAT AACTATAAGG TCCATCTTTT
551   ATATGTTCTG CCTGAAGTGT TAGAAGATTC ACCTCTGGTT CCCCAAAAAG
601   GCAGTTTTCA GATGGTTCAC TGCAATTGCA GTGTTCATGA ATGTTGTGAA
651   TGTCTTGTGC CTGTGCCAAC AGCCAAACTC AACGACACTC TCCTTATGTG
701   TTTGAAAATC ACATCTGGTG GAGTAATTTT CCAGTCACCT CTAATGTCAG
751   TTCAGCCCAT AAATATGGTG AAGCCTGATC CACCATTAGG TTTGCATATG
801   GAAATCACAG ATGATGGTAA TTTAAAGATT TCTTGGTCCA GCCCACCATT
851   GGTACCATTT CCACTTCAAT ATCAAGTGAA ATATTCAGAG AATTCTACAA
901   CAGTTATCAG AGAAGCTGAC AAGATTGTCT CAGCTACATC CCTGCTAGTA
951   GACAGTATAC TTCCTGGGTC TTCGTATGAG GTTCAGGTGA GGGGCAAGAG
1001  ACTGGATGGC CCAGGAATCT GGAGTGACTG GAGTACTCCT CGTGTCTTTA
1051  CCACACAAGA TGTCATATAC TTTCCACCTA AAATTCTGAC AAGTGTTGGG
1101  TCTAATGTTT CTTTTCACTG CATCTATAAG AAGGAAAACA AGATTGTTCC
1151  CTCAAAAGAG ATTGTTTGGT GGATGAATTT AGCTGAGAAA ATTCCTCAAA
1201  GCCAGTATGA TGTTGTGAGT GATCATGTTA GCAAAGTTAC TTTTTTCAAT
1251  CTGAATGAAA CCAAACCTCG AGGAAAGTTT ACCTATGATG CAGTGTACTG
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1301 CTGCAATGAA CATGAATGCC ATCATCGCTA TGCTGAATTA TATGTGATTG
1351 ATGTCAATAT CAATATCTCA TGTGAAACTG ATGGGTACTT AACTAAAATG
1401 ACTTGCAGAT GGTCAACCAG TACAATCCAG TCACTTGCGG AAAGCACTTT
1451 GCAATTGAGG TATCATAGGA GCAGCCTTTA CTGTTCTGAT ATTCCATCTA
1501 TTCATCCCAT ATCTGAGCCC AAAGATTGCT ATTTGCAGAG TGATGGTTTT
1551 TATGAATGCA TTTTCCAGCC AATCTTCCTA TTATCTGGCT ACACAATGTG
1601 GATTAGGATC AATCACTCTC TAGGTTCACT TGACTCTCCA CCAACATGTG
1651 TCCTTCCTGA TTCTGTGGTG AAGCCACTGC CTCCATCCAG TGTGAAAGCA
1701 GAAATTACTA TAAACATTGG ATTATTGAAA ATATCTTGGG AAAAGCCAGT
1751 CTTTCCAGAG AATAACCTTC AATTCCAGAT TCGCTATGGT TTAAGTGGAA
1801 AAGAAGTACA ATGGAAGATG TATGAGGTTT ATGATGCAAA ATCAAAATCT
1851 GTCAGTCTCC CAGTTCCAGA CTTGTGTGCA GTCTATGCTG TTCAGGTGCG
1901 CTGTAAGAGG CTAGATGGAC TGGGATATTG GAGTAATTGG AGCAATCCAG
1951 CCTACACAGT TGTCATGGAT ATAAAAGTTC CTATGAGAGG ACCTGAATTT
2001 TGGAGAATAA TTAATGGAGA TACTATGAAA AAGGAGAAAA ATGTCACTTT
2051 ACTTTGGAAG CCCCTGATGA AAAATGACTC ATTGTGCAGT GTTCAGAGAT
2101 ATGTGATAAA CCATCATACT TCCTGCAATG GAACATGGTC AGAAGATGTG
2151 GGAAATCACA CGAAATTCAC TTTCCTGTGG ACAGAGCAAG CACATACTGT
2201 TACGGTTCTG GCCATCAATT CAATTGGTGC TTCTGTTGCA AATTTTAATT
2251 TAACCTTTTC ATGGCCTATG AGCAAAGTAA ATATCGTGCA GTCACTCAGT
2301 GCTTATCCTT TAAACAGCAG TTGTGTGATT GTTTCCTGGA TACTATCACC
2351 CAGTGATTAC AAGCTAATGT ATTTTATTAT TGAGTGGAAA AATCTTAATG
2401 AAGATGGTGA AATAAAATGG CTTAGAATCT CTTCATCTGT TAAGAAGTAT
2451 TATATCCATG ATCATTTTAT CCCCATTGAG AAGTACCAGT TCAGTCTTTA
2501 CCCAATATTT ATGGAAGGAG TGGGAAAACC AAAGATAATT AATAGTTTCA
2551 CTCAAGATGA TATTGAAAAA CACCAGAGTG ATGCAGGTTT ATATGTAATT

2601 GTGCCAGTAA TTATTTCCTC TTCCATCTTA TTGCTTGGAA CATTATTAAT

2651 ATCACACCAA AGAATGAAAA AGCTATTTTG GGAAGATGTT CCGAACCCCA

2701 AGAATTGTTC CTGGGCACAA GGACTTAATT TTCAGAAGAG AACGGACATT

2751 CTTTGAAGTC TAATCATGAT CACTACAGAT GAACCCAATG TGCCAACTTC

2801 CCAACAGTCT ATAGAGTATT AGAAGATTTT TACATTTTGA AGAAGGGGAG

2851 CAAATCTAAA AAAAATTCAG TTGAACTTCT GAGAGTTAAC ATATGGTGGA

2901 TTATGTTGAT TTAGAACTTA AAATAGATGT GTAAATTTGG GTTCAAAATG

2951 TAGATTTGAG TCCAGTTTGG ATGTGTGATT AATTTTCAAA TCATCTAAAG

3001 TTTAAAAGTA GTATTCATGA TTTCTGGCTT TTGATTTGCC ATATTCCTGG

3051 TCATAAAACA TTAAGAAAAT TATGGCTGTT GCTGTCATTA CATATCTATT

3101 AAATGTCATC AAATATGTAG TAGACAATTT TGTAATTAGG TGAACTCTAA

3151 AACTGCAACA TCTGACAAAT TGCTTTAAAA ATACAATGAT TAT

Human OB Receptor "B" Amino Acid Sequence (Seq. ID No. 3 (Amino Acid)):

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1    MICQKFCVVL LHWEFIYVIT AFNLSYPITP WRFKLSCMPP NSTYDYFLLP
51   AGLSKNTSNS NGHYETAVEP KFNSSGTHFS NLSKTTFHCC FRSEQDRNCS
101  LCADNIEGKT FVSTVNSLVF QQIDANWNIQ CWLKGDLKLF ICYVESLFKN
151  LFRNYNYKVH LLYVLPEVLE DSPLVPQKGS FQMVHCNCSV HECCECLVPV
201  PTAKLNDTLL MCLKITSGGV IFQSPLMSVQ PINMVKPDPP LGLHMEITDD
251  GNLKISWSSP PLVPFPLQYQ VKYSENSTTV IREADKIVSA TSLLVDSILP
301  GSSYEVQVRG KRLDGPGIWS DWSTPRVFTT QDVIYFPPKI LTSVGSNVSF
351  HCIYKKENKI VPSKEIVWWM NLAEKIPQSQ YDVVSDHVSK VTFFNLNETK
401  PRGKFTYDAV YCCNEHECHH RYAELYVIDV NINISCETDG YLTKMTCRWS
451  TSTIQSLAES TLQLRYHRSS LYCSDIPSIH PISEPKDCYL QSDGFYECIF
501  QPIFLLSGYT MWIRINHSLG SLDSPPTCVL PDSVVKPLPP SSVKAEITIN
551  IGLLKISWEK PVFPENNLQF QIRYGLSGKE VQWKMYEVYD AKSKSVSLPV
601  PDLCAVYAVQ VRCKRLDGLG YWSNWSNPAY TVVMDIKVPM RGPEFWRIIN
651  GDTMKKEKNV TLLWKPLMKN DSLCSVQRYV INHHTSCNGT WSEDVGNHTK
701  FTFLWTEQAH TVTVLAINSI GASVANFNLT FSWPMSKVNI VQSLSAYPLN
751  SSCVIVSWIL SPSDYKLMYF IIEWKNLNED GEIKWLRISS SVKKYYIHDH
801  FIPIEKYQFS LYPIFMEGVG KPKIINSFTQ DDIEKHQSDA GLYVIVPVII
851  SSSILLLGTL LISHQRMKKL FWEDVPNPKN CSWAQGLNFQ KKRLSIFLSS
901  IQHQ*HVVLF FWSLKQFQKI SVLIHHGKIK MR*CQQLWSL YFQQQILKRV
951  LFVLVTSSTV LTSLRLRVLR *PMRTKARDN PLLNTPR*SA TLNQVKLVK
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Human OB Receptor "B" DNA Sequence (Seq. ID No. 4 (DNA)):

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1     CCGCCGCCAT CTCTGCCTTC GGTCGAGTTG GACCCCCGGA TCAAGGTGTA
51    CTTCTCTGAA GTAAGATGAT TTGTCAAAAA TTCTGTGTGG TTTTGTTACA
101   TTGGGAATTT ATTTATGTGA TAACTGCGTT TAACTTGTCA TATCCAATTA
151   CTCCTTGGAG ATTTAAGTTG TCTTGCATGC CACCAAATTC AACCTATGAC
201   TACTTCCTTT TGCCTGCTGG ACTCTCAAAG AATACTTCAA ATTCGAATGG
251   ACATTATGAG ACAGCTGTTG AACCTAAGTT TAATTCAAGT GGTACTCACT
301   TTTCTAACTT ATCCAAAACA ACTTTCCACT GTTGCTTTCG GAGTGAGCAA
351   GATAGAAACT GCTCCTTATG TGCAGACAAC ATTGAAGGAA AGACATTTGT
401   TTCAACAGTA AATTCTTTAG TTTTTCAACA AATAGATGCA AACTGGAACA
451   TACAGTGCTG GCTAAAAGGA GACTTAAAAT TATTCATCTG TTATGTGGAG
501   TCATTATTTA AGAATCTATT CAGGAATTAT AACTATAAGG TCCATCTTTT
551   ATATGTTCTG CCTGAAGTGT TAGAAGATTC ACCTCTGGTT CCCCAAAAAG
601   GCAGTTTTCA GATGGTTCAC TGCAATTGCA GTGTTCATGA ATGTTGTGAA
651   TGTCTTGTGC CTGTGCCAAC AGCCAAACTC AACGACACTC TCCTTATGTG
701   TTTGAAAATC ACATCTGGTG GAGTAATTTT CCAGTCACCT CTAATGTCAG
751   TTCAGCCCAT AAATATGGTG AAGCCTGATC CACCATTAGG TTTGCATATG
801   GAAATCACAG ATGATGGTAA TTTAAAGATT TCTTGGTCCA GCCCACCATT
851   GGTACCATTT CCACTTCAAT ATCAAGTGAA ATATTCAGAG AATTCTACAA
901   CAGTTATCAG AGAAGCTGAC AAGATTGTCT CAGCTACATC CCTGCTAGTA
951   GACAGTATAC TTCCTGGGTC TTCGTATGAG GTTCAGGTGA GGGGCAAGAG
1001  ACTGGATGGC CCAGGAATCT GGAGTGACTG GAGTACTCCT CGTGTCTTTA
1051  CCACACAAGA TGTCATATAC TTTCCACCTA AAATTCTGAC AAGTGTTGGG
1101  TCTAATGTTT CTTTTCACTG CATCTATAAG AAGGAAAACA AGATTGTTCC
1151  CTCAAAAGAG ATTGTTTGGT GGATGAATTT AGCTGAGAAA ATTCCTCAAA
1201  GCCAGTATGA TGTTGTGAGT GATCATGTTA GCAAAGTTAC TTTTTTCAAT
1251  CTGAATGAAA CCAAACCTCG AGGAAAGTTT ACCTATGATG CAGTGTACTG
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1301 CTGCAATGAA CATGAATGCC ATCATCGCTA TGCTGAATTA TATGTGATTG
1351 ATGTCAATAT CAATATCTCA TGTGAAACTG ATGGGTACTT AACTAAAATG
1401 ACTTGCAGAT GGTCAACCAG TACAATCCAG TCACTTGCGG AAAGCACTTT
1451 GCAATTGAGG TATCATAGGA GCAGCCTTTA CTGTTCTGAT ATTCCATCTA
1501 TTCATCCCAT ATCTGAGCCC AAAGATTGCT ATTTGCAGAG TGATGGTTTT
1551 TATGAATGCA TTTTCCAGCC AATCTTCCTA TTATCTGGCT ACACAATGTG
1601 GATTAGGATC AATCACTCTC TAGGTTCACT TGACTCTCCA CCAACATGTG
1651 TCCTTCCTGA TTCTGTGGTG AAGCCACTGC CTCCATCCAG TGTGAAAGCA
1701 GAAATTACTA TAAACATTGG ATTATTGAAA ATATCTTGGG AAAAGCCAGT
1751 CTTTCCAGAG AATAACCTTC AATTCCAGAT TCGCTATGGT TTAAGTGGAA
1801 AAGAAGTACA ATGGAAGATG TATGAGGTTT ATGATGCAAA ATCAAAATCT
1851 GTCAGTCTCC CAGTTCCAGA CTTGTGTGCA GTCTATGCTG TTCAGGTGCG
1901 CTGTAAGAGG CTAGATGGAC TGGGATATTG GAGTAATTGG AGCAATCCAG
1951 CCTACACAGT TGTCATGGAT ATAAAAGTTC CTATGAGAGG ACCTGAATTT
2001 TGGAGAATAA TTAATGGAGA TACTATGAAA AAGGAGAAAA ATGTCACTTT
2051 ACTTTGGAAG CCCCTGATGA AAAATGACTC ATTGTGCAGT GTTCAGAGAT
2101 ATGTGATAAA CCATCATACT TCCTGCAATG GAACATGGTC AGAAGATGTG
2151 GGAAATCACA CGAAATTCAC TTTCCTGTGG ACAGAGCAAG CACATACTGT
2201 TACGGTTCTG GCCATCAATT CAATTGGTGC TTCTGTTGCA AATTTTAATT
2251 TAACCTTTTC ATGGCCTATG AGCAAAGTAA ATATCGTGCA GTCACTCAGT
2301 GCTTATCCTT TAAACAGCAG TTGTGTGATT GTTTCCTGGA TACTATCACC
2351 CAGTGATTAC AAGCTAATGT ATTTTATTAT TGAGTGGAAA AATCTTAATG
2401 AAGATGGTGA AATAAAATGG CTTAGAATCT CTTCATCTGT TAAGAAGTAT
2451 TATATCCATG ATCATTTTAT CCCCATTGAG AAGTACCAGT TCAGTCTTTA
2501 CCCAATATTT ATGGAAGGAG TGGGAAAACC AAAGATAATT AATAGTTTCA
2551 CTCAAGATGA TATTGAAAAA CACCAGAGTG ATGCAGGTTT ATATGTAATT

2601 GTGCCAGTAA TTATTTCCTC TTCCATCTTA TTGCTTGGAA CATTATTAAT

2651 ATCACACCAA AGAATGAAAA AGCTATTTTG GGAAGATGTT CCGAACCCCA

2701 AGAATTGTTC CTGGGCACAA GGACTTAATT TTCAGAAGAA ACGTTTGAGC

2751 ATCTTTTTAT CAAGCATACA GCATCAGTGA CATGTGGTCC TCTTCTTTTG

2801 GAGCCTGAAA CAATTTCAGA AGATATCAGT GTTGATACAT CATGGAAAAA

2851 TAAAGATGAG ATGATGCCAA CAACTGTGGT CTCTCTACTT TCAACAACAG

2901 ATCTTGAAAA GGGTTCTGTT TGTTTTAGTG ACCAGTTCAA CAGTGTTAAC

2951 TTCTCTGAGG CTGAGGGTAC TGAGGTAACC TATGAGGACG AAAGCCAGAG

3001 ACAACCCTTT GTTAAATACG CCACGCTGAT CAGCAACTCT AAACCAAGTG

3051 AAACTGGTGA AGA

Human OB Receptor "C" Amino Acid Sequence (Seq. ID No. 5 (Amino Acid)):

1 MICQKFCVVL LHWEFIYVIT AFNLSYPITP WRFKLSCMPP NSTYDYFLLP
51 AGLSKNTSNS NGHYETAVEP KFNSSGTHFS NLSKTTFHCC FRSEQDRNCS
101 LCADNIEGKT FVSTVNSLVF QQIDANWNIQ CWLKGDLKLF ICYVESLFKN
151 LFRNYNYKVH LLYVLPEVLE DSPLVPQKGS FQMVHCNCSV HECCECLVPV
201 PTAKLNDTLL MCLKITSGGV IFQSPLMSVQ PINMVKPDPP LGLHMEITDD
251 GNLKISWSSP PLVPFPLQYQ VKYSENSTTV IREADKIVSA TSLLVDSILP
301 GSSYEVQVRG KRLDGPGIWS DWSTPRVFTT QDVIYFPPKI LTSVGSNVSF
351 HCIYKKENKI VPSKEIVWWM NLAEKIPQSQ YDVVSDHVSK VTFFNLNETK
401 PRGKFTYDAV YCCNEHECHH RYAELYVIDV NINISCETDG YLTKMTCRWS
451 TSTIQSLAES TLQLRYHRSS LYCSDIPSIH PISEPKDCYL QSDGFYECIF
501 QPIFLLSGYT MWIRINHSLG SLDSPPTCVL PDSVVKPLPP SSVKAEITIN
551 IGLLKISWEK PVFPENNLQF QIRYGLSGKE VQWKMYEVYD AKSKSVSLPV
601 PDLCAVYAVQ VRCKRLDGLG YWSNWSNPAY TVVMDIKVPM RGPEFWRIIN
651 GDTMKKEKNV TLLWKPLMKN DSLCSVQRYV INHHTSCNGT WSEDVGNHTK
701 FTFLWTEQAH TVTVLAINSI GASVANFNLT FSWPMSKVNI VQSLSAYPLN
751 SSCVIVSWIL SPSDYKLMYF IIEWKNLNED GEIKWLRISS SVKKYYIHDH
801 FIPIEKYQFS LYPIFMEGVG KPKIINSFTQ DDIEKHQSDA GLYVIVPVII
851 SSSILLLGTL LISHQRMKKL FWEDVPNPKN CSWAQGLNFQ KMLEGSMFVK
901 SHHHSLISST QGHKHCGRPQ GPLHRKTRDL CSLVYLLTLP PLLSYDPAKS
951 PSVRNTQE*S IKKKKKKLEG

Human OB Receptor "C" DNA Sequence (Seq. ID No. 6 (DNA)):

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1     CCGCCGCCAT CTCTGCCTTC GGTCGAGTTG GACCCCCGGA TCAAGGTGTA
51    CTTCTCTGAA GTAAGATGAT TTGTCAAAAA TTCTGTGTGG TTTTGTTACA
101   TTGGGAATTT ATTTATGTGA TAACTGCGTT TAACTTGTCA TATCCAATTA
151   CTCCTTGGAG ATTTAAGTTG TCTTGCATGC CACCAAATTC AACCTATGAC
201   TACTTCCTTT TGCCTGCTGG ACTCTCAAAG AATACTTCAA ATTCGAATGG
251   ACATTATGAG ACAGCTGTTG AACCTAAGTT TAATTCAAGT GGTACTCACT
301   TTTCTAACTT ATCCAAAACA ACTTTCCACT GTTGCTTTCG GAGTGAGCAA
351   GATAGAAACT GCTCCTTATG TGCAGACAAC ATTGAAGGAA AGACATTTGT
401   TTCAACAGTA AATTCTTTAG TTTTTCAACA AATAGATGCA AACTGGAACA
451   TACAGTGCTG GCTAAAAGGA GACTTAAAAT TATTCATCTG TTATGTGGAG
501   TCATTATTTA AGAATCTATT CAGGAATTAT AACTATAAGG TCCATCTTTT
551   ATATGTTCTG CCTGAAGTGT TAGAAGATTC ACCTCTGGTT CCCCAAAAAG
601   GCAGTTTTCA GATGGTTCAC TGCAATTGCA GTGTTCATGA ATGTTGTGAA
651   TGTCTTGTGC CTGTGCCAAC AGCCAAACTC AACGACACTC TCCTTATGTG
701   TTTGAAAATC ACATCTGGTG GAGTAATTTT CCAGTCACCT CTAATGTCAG
751   TTCAGCCCAT AAATATGGTG AAGCCTGATC CACCATTAGG TTTGCATATG
801   GAAATCACAG ATGATGGTAA TTTAAAGATT TCTTGGTCCA GCCCACCATT
851   GGTACCATTT CCACTTCAAT ATCAAGTGAA ATATTCAGAG AATTCTACAA
901   CAGTTATCAG AGAAGCTGAC AAGATTGTCT CAGCTACATC CCTGCTAGTA
951   GACAGTATAC TTCCTGGGTC TTCGTATGAG GTTCAGGTGA GGGGCAAGAG
1001  ACTGGATGGC CCAGGAATCT GGAGTGACTG GAGTACTCCT CGTGTCTTTA
1051  CCACACAAGA TGTCATATAC TTTCCACCTA AAATTCTGAC AAGTGTTGGG
1101  TCTAATGTTT CTTTTCACTG CATCTATAAG AAGGAAAACA AGATTGTTCC
1151  CTCAAAAGAG ATTGTTTGGT GGATGAATTT AGCTGAGAAA ATTCCTCAAA
1201  GCCAGTATGA TGTTGTGAGT GATCATGTTA GCAAAGTTAC TTTTTTCAAT
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1251 CTGAATGAAA CCAAACCTCG AGGAAAGTTT ACCTATGATG CAGTGTACTG

1301 CTGCAATGAA CATGAATGCC ATCATCGCTA TGCTGAATTA TATGTGATTG

1351 ATGTCAATAT CAATATCTCA TGTGAAACTG ATGGGTACTT AACTAAAATG

1401 ACTTGCAGAT GGTCAACCAG TACAATCCAG TCACTTGCGG AAAGCACTTT

1451 GCAATTGAGG TATCATAGGA GCAGCCTTTA CTGTTCTGAT ATTCCATCTA

1501 TTCATCCCAT ATCTGAGCCC AAAGATTGCT ATTTGCAGAG TGATGGTTTT

1551 TATGAATGCA TTTTCCAGCC AATCTTCCTA TTATCTGGCT ACACAATGTG

1601 GATTAGGATC AATCACTCTC TAGGTTCACT TGACTCTCCA CCAACATGTG

1651 TCCTTCCTGA TTCTGTGGTG AAGCCACTGC CTCCATCCAG TGTGAAAGCA

1701 GAAATTACTA TAAACATTGG ATTATTGAAA ATATCTTGGG AAAAGCCAGT

1751 CTTTCCAGAG AATAACCTTC AATTCCAGAT TCGCTATGGT TTAAGTGGAA

1801 AAGAAGTACA ATGGAAGATG TATGAGGTTT ATGATGCAAA ATCAAAATCT

1851 GTCAGTCTCC CAGTTCCAGA CTTGTGTGCA GTCTATGCTG TTCAGGTGCG

1901 CTGTAAGAGG CTAGATGGAC TGGGATATTG GAGTAATTGG AGCAATCCAG

1951 CCTACACAGT TGTCATGGAT ATAAAAGTTC CTATGAGAGG ACCTGAATTT

2001 TGGAGAATAA TTAATGGAGA TACTATGAAA AAGGAGAAAA ATGTCACTTT

2051 ACTTTGGAAG CCCCTGATGA AAAATGACTC ATTGTGCAGT GTTCAGAGAT

2101 ATGTGATAAA CCATCATACT TCCTGCAATG GAACATGGTC AGAAGATGTG

2151 GGAAATCACA CGAAATTCAC TTTCCTGTGG ACAGAGCAAG CACATACTGT

2201 TACGGTTCTG GCCATCAATT CAATTGGTGC TTCTGTTGCA AATTTTAATT

2251 TAACCTTTTC ATGGCCTATG AGCAAAGTAA ATATCGTGCA GTCACTCAGT

2301 GCTTATCCTT TAAACAGCAG TTGTGTGATT GTTTCCTGGA TACTATCACC

2351 CAGTGATTAC AAGCTAATGT ATTTTATTAT TGAGTGGAAA AATCTTAATG

2401 AAGATGGTGA AATAAAATGG CTTAGAATCT CTTCATCTGT TAAGAAGTAT

2451 TATATCCATG ATCATTTTAT CCCCATTGAG AAGTACCAGT TCAGTCTTTA

2501 CCCAATATTT ATGGAAGGAG TGGGAAAACC AAAGATAATT AATAGTTTCA

2551 CTCAAGATGA TATTGAAAAA CACCAGAGTG ATGCAGGTTT ATATGTAATT

2601 GTGCCAGTAA TTATTTCCTC TTCCATCTTA TTGCTTGGAA CATTATTAAT

2651 ATCACACCAA AGAATGAAAA AGCTATTTTG GGAAGATGTT CCGAACCCCA

2701 AGAATTGTTC CTGGGCACAA GGACTTAATT TTCAGAAGAT GCTTGAAGGC

2751 AGCATGTTCG TTAAGAGTCA TCACCACTCC CTAATCTCAA GTACCCAGGG

2801 ACACAAACAC TGCGGAAGGC CACAGGGTCC TCTGCATAGG AAAACCAGAG

2851 ACCTTTGTTC ACTTGTTTAT CTGCTGACCC TCCCTCCACT ATTGTCCTAT

2901 GACCCTGCCA AATCCCCCTC TGTGAGAAAC ACCCAAGAAT GATCAATAAA

2951 AAAAAAAAAA AAAAAACTCG AGGGGG

Human OB Receptor "D" Amino Acid Sequence (Sequence ID No. 7)

```
    1  MICQKFCVVL LHWEFIYVIT AFNLSYPITP WRFKLSCMPP NSTYDYFLLP
   51  AGLSKNTSNS NGHYETAVEP KFNSSGTHFS NLSKTTFHCC FRSEQDRNCS
  101  LCADNIEGKT FVSTVNSLVF QQIDANWNIQ CWLKGDLKLF ICYVESLFKN
  151  LFRNYNYKVH LLYVLPEVLE DSPLVPQKGS FQMVHCNCSV HECCECLVPV
  201  PTAKLNDTLL MCLKITSGGV IFQSPLMSVQ PINMVKPDPP LGLHMEITDD
  251  GNLKISWSSP PLVPFPLQYQ VKYSENSTTV IREADKIVSA TSLLVDSILP
  301  GSSYEVQVRG KRLDGPGIWS DWSTPRVFTT QDVIYFPPKI LTSVGSNVSF
  351  HCIYKKENKI VPSKEIVWWM NLAEKIPQSQ YDVVSDHVSK VTFFNLNETK
  401  PRGKFTYDAV YCCNEHECHH RYAELYVIDV NINISCETDG YLTKMTCRWS
  451  TSTIQSLAES TLQLRYHRSS LYCSDIPSIH PISEPKDCYL QSDGFYECIF
  501  QPIFLLSGYT MWIRINHSLG SLDSPPTCVL PDSVVKPLPP SSVKAEITIN
  551  IGLLKISWEK PVFPENNLQF QIRYGLSGKE VQWKMYEVYD AKSKSVSLPV
  601  PDLCAVYAVQ VRCKRLDGLG YWSNWSNPAY TVVMDIKVPM RGPEFWRIIN
  651  GDTMKKEKNV TLLWKPLMKN DSLCSVQRYV INHHTSCNGT WSEDVGNHTK
  701  FTFLWTEQAH TVTVLAINSI GASVANFNLT FSWPMSKVNI VQSLSAYPLN
  751  SSCVIVSWIL SPSDYKLMYF IIEWKNLNED GEIKWLRISS SVKKYYIHDH
  801  FIPIEKYQFS LYPIFMEGVG KPKIINSFTQ DDIEKHQSDA GLYVIVPVII
  851  SSSILLLGTL LISHQRMKKL FWEDVPNPKN CSWAQGLNFQ KPETFEHLFI
  901  KHTASVTCGP LLLEPETISE DISVDTSWKN KDEMMPTTVV SLLSTTDLEK
  951  GSVCISDQFN SVNFSEAEGT EVTYEDESQR QPFVKYATLI SNSKPSETGE
 1001  EQGLINSSVT KCFSSKNSPL KDSFSNSSWE IEAQAFFILS DQHPNIISPH
 1051  LTFSEGLDEL LKLEGNFPEE NNDKKSIYYL GVTSIKKRES GVLLTDKSRV
 1101  SCPFPAPCLF TDIRVLQDSC SHFVENNINL GTSSKKTFAS YMPQFQTCST
 1151  QTHKIMENKM CDLTV*FH*R NLQICVIMGN IKCNRL*LWV GERKETRVKF
 1201  ENNCSK*KKK KKNSRPARPD
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Human OB Receptor "D" Nucleic Acid Sequence (Sequence ID No. 8)

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1     GCGGCCGCCA GTGTGATGGA TATCTGCAGA ATTCGGCTTT CTCTGCCTTC
51    GGTCGAGTTG GACCCCCGGA TCAAGGTGTA CTTCTCTGAA GTAAGATGAT
101   TTGTCAAAAA TTCTGTGTGG TTTTGTTACA TTGGGAATTT ATTTATGTGA
151   TAACTGCGTT TAACTTGTCA TATCCAATTA CTCCTTGGAG ATTTAAGTTG
201   TCTTGCATGC CACCAAATTC AACCTATGAC TACTTCCTTT TGCCTGCTGG
251   GCTCTCAAAG AATACTTCAA ATTCGAATGG ACATTATGAG ACAGCTGTTG
301   AACCTAAGTT TAATTCAAGT GGTACTCACT TTTCTAACTT ATCCAAAACA
351   ACTTTCCACT GTTGCTTTCG GAGTGAGCAA GATAGAAACT GCTCCTTATG
401   TGCAGACAAC ATTGAAGGAA AGACATTTGT TTCAACAGTA AATTCTTTAG
451   TTTTTCAACA AATAGATGCA AACTGGAACA TACAGTGCTG GCTAAAAGGA
501   GACTTAAAAT TATTCATCTG TTATGTGGAG TCATTATTTA AGAATCTATT
551   CAGGAATTAT AACTATAAGG TCCATCTTTT ATATGTTCTG CCTGAAGTGT
601   TAGAAGATTC ACCTCTGGTT CCCCAAAAAG GCAGTTTTCA GATGGTTCAC
651   TGCAATTGCA GTGTTCACGA ATGTTGTGAA TGTCTTGTGC CTGTGCCAAC
701   AGCCAAACTC AACGACACTC TCCTTATGTG TTTGAAAATC ACATCTGGTG
751   GAGTAATTTT CCAGTCACCT CTAATGTCAG TTCAGCCCAT AAATATGGTG
801   AAGCCTGATC CACCATTAGG TTTGCATATG GAAATCACAG ATGATGGTAA
851   TTTAAAGATT TCTTGGTCCA GCCCACCATT GGTACCATTT CCACTTCAAT
901   ATCAAGTGAA ATATTCAGAG AATTCTACAA CAGTTATCAG AGAAGCTGAC
951   AAGATTGTCT CAGCTACATC CCTGCTAGTA GACAGTATAC TTCCTGGGTC
1001  TTCGTATGAG GTTCAGGTGA GGGGCAAGAG ACTGGATGGC CCAGGAATCT
1051  GGAGTGACTG GAGTACTCCT CGTGTCTTTA CCACACAAGA TGTCATATAC
1101  TTTCCACCTA AAATTCTGAC AAGTGTTGGG TCTAATGTTT CTTTTCACTG
1151  CATCTATAAG AAGGAAAACA AGATTGTTCC CTCAAAAGAG ATTGTTTGGT
1201  GGATGAATTT AGCTGAGAAA ATTCCTCAAA GCCAGTATGA TGTTGTGAGT
1251  GATCATGTTA GCAAAGTTAC TTTTTTCAAT CTGAATGAAA CCAAACCTCG
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1301 AGGAAAGTTT ACCTATGATG CAGTGTACTG CTGCAATGAA CATGAATGCC

1351 ATCATCGCTA TGCTGAATTA TATGTGATTG ATGTCAATAT CAATATCTCA

1401 TGTGAAACTG ATGGGTACTT AACTAAAATG ACTTGCAGAT GGTCAACCAG

1451 TACAATCCAG TCACTTGCGG AAAGCACTTT GCAATTGAGG TATCATAGGA

1501 GCAGCCTTTA CTGTTCTGAT ATTCCATCTA TTCATCCCAT ATCTGAGCCC

1551 AAAGATTGCT ATTTGCAGAG TGATGGTTTT TATGAATGCA TTTTCCAGCC

1601 AATCTTCCTA TTATCTGGCT ACACAATGTG GATTAGGATC AATCACTCTC

1651 TAGGTTCACT TGACTCTCCA CCAACATGTG TCCTTCCTGA TTCTGTGGTG

1701 AAGCCACTGC CTCCATCCAG TGTGAAAGCA GAAATTACTA TAAACATTGG

1751 ATTATTGAAA ATATCTTGGG AAAAGCCAGT CTTTCCAGAG AATAACCTTC

1801 AATTCCAGAT TCGCTATGGT TTAAGTGGAA AAGAAGTACA ATGGAAGATG

1851 TATGAGGTTT ATGATGCAAA ATCAAAATCT GTCAGTCTCC CAGTTCCAGA

1901 CTTGTGTGCA GTCTATGCTG TTCAGGTGCG CTGTAAGAGG CTAGATGGAC

1951 TGGGATATTG GAGTAATTGG AGCAATCCAG CCTACACAGT TGTCATGGAT

2001 ATAAAAGTTC CTATGAGAGG ACCTGAATTT TGGAGAATAA TTAATGGAGA

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2101 AAAATGACTC ATTGTGCAGT GTTCAGAGAT ATGTGATAAA CCATCATACT

2151 TCCTGCAATG GAACATGGTC AGAAGATGTG GGAAATCACA CGAAATTCAC

2201 TTTCCTGTGG ACAGAGCAAG CACATACTGT TACGGTTCTG GCCATCAATT

2251 CAATTGGTGC TTCTGTTGCA AATTTTAATT TAACCTTTTC ATGGCCTATG

2301 AGCAAAGTAA ATATCGTGCA GTCACTCAGT GCTTATCCTT TAAACAGCAG

2351 TTGTGTGATT GTTTCCTGGA TACTATCACC CAGTGATTAC AAGCTAATGT

2401 ATTTTATTAT TGAGTGGAAA AATCTTAATG AAGATGGTGA AATAAAATGG

2451 CTTAGAATCT CTTCATCTGT TAAGAAGTAT TATATCCATG ATCATTTTAT

2501 CCCCATTGAG AAGTACCAGT TCAGTCTTTA CCCAATATTT ATGGAAGGAG

2551 TGGGAAAACC AAAGATAATT AATAGTTTCA CTCAAGATGA TATTGAAAAA

2601 CACCAGAGTG ATGCAGGTTT ATATGTAATT GTGCCAGTAA TTATTTCCTG

2651 TTCCATCTTA TTGCTTGGAA CATTATTAAT ATCACACCAA AGAATGAAAA

2701 AGCTATTTTG GGAAGATGTT CCGAACCCCA AGAATTGTTC CTGGGCACAA

2751 GGACTTAATT TTCAGAAGCC AGAAACGTTT GAGCATCTTT TTATCAAGCA

2801 TACAGCATCA GTGACATGTG GTCCTCTTCT TTTGGAGCCT GAAACAATTT

2851 CAGAAGATAT CAGTGTTGAT ACATCATGGA AAAATAAAGA TGAGATGATG

2901 CCAACAACTG TGGTCTCTCT ACTTTCAACA ACAGATCTTG AAAAGGGTTC

2951 TGTTTGTATT AGTGACCAGT TCAACAGTGT TAACTTCTCT GAGGCTGAGG

3001 GTACTGAGGT AACCTATGAG GACGAAAGCC AGAGACAAGC CTTTGTTAAA

3051 TACGCCACGC TGATCAGCAA CTCTAAACCA AGTGAAACTG GTGAAGAACA

3101 AGGGCTTATA AATAGTTCAG TCACCAAGTG CTTCTCTAGC AAAAATTCTG

3151 CGTTGAAGGA TTCTTTCTCT AATAGCTCAT GGGAGATAGA GGCCCAGGCA

3201 TTTTTTATAT TATCGGATCA GCATCCCAAC ATAATTTCAC CACACCTCAC

3251 ATTCTCAGAA GGATTGGATG AACTTTTGAA ATTGGAGGGA AATTTCCCTG

3301 AAGAAAATAA TGATAAAAAG TCTATCTATT ATTTAGGGGT CACCTCAATG

3351 AAAAAGAGAG AGAGTGGTGT GCTTTTGACT GACAAGTCAA GGGTATCGTG

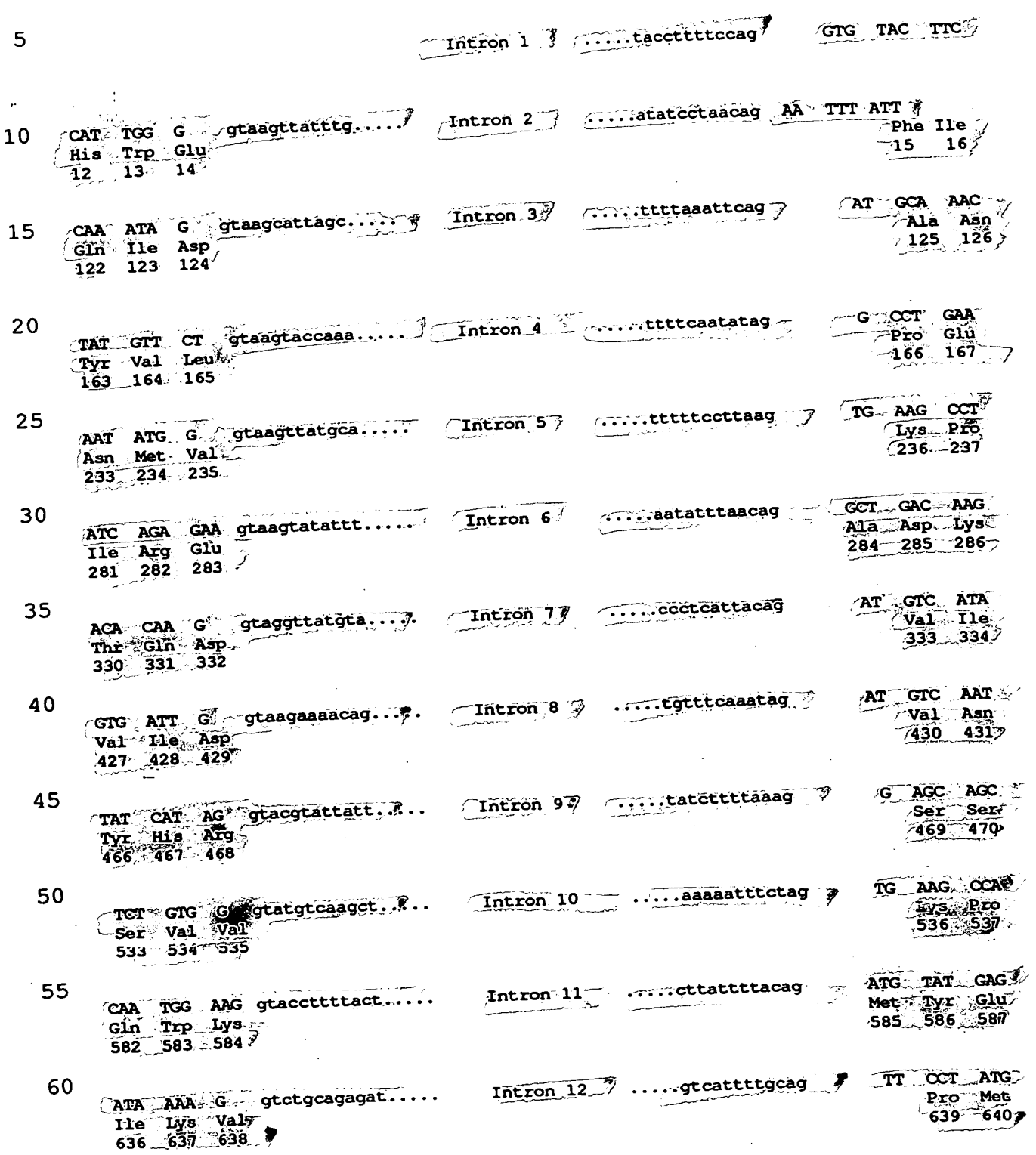
3401 CCCATTCCCA GCCCCCTGTT TATTCACGGA CATCAGAGTT CTCCAGGACA

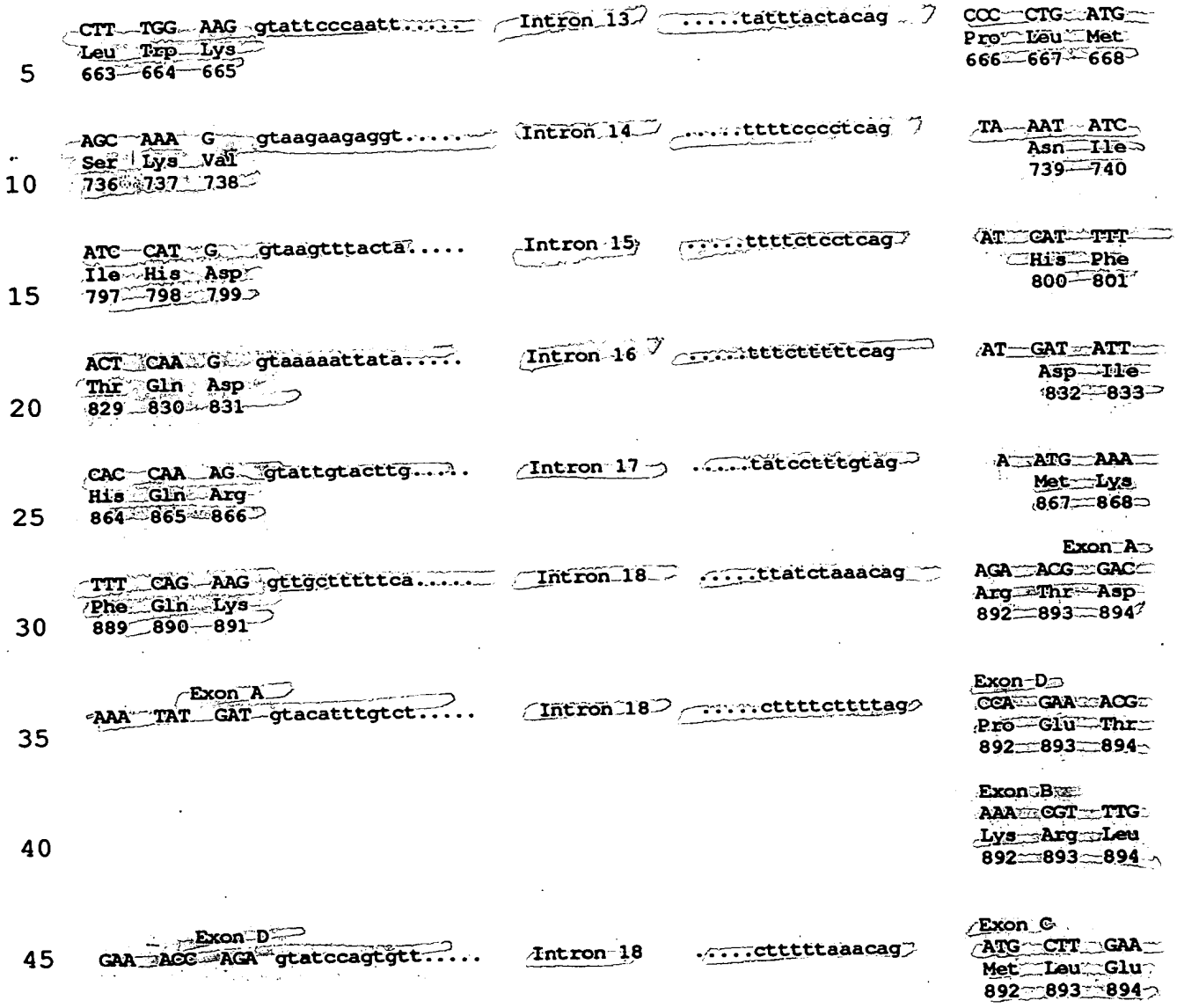
3451 GTTGCTCACA CTTTGTAGAA AATAATATCA ACTTAGGAAC TTCTAGTAAG

3501 AAGACTTTTG CATCTTACAT GCCTCAATTC CAAACTTGTT CTACTCAGAC

3551 TCATAAGATC ATGGAAAACA AGATGTGTGA CCTAACTGTG TAATCTAGA

Human OB Receptor Protein "D" Chromosomal DNA (Seq. ID No. 9)

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Leu Trp Lys
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Intron 13
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CCC CTG ATG
Pro Leu Met
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AGC AAA G gtaagaagaggt.....
Ser Lys Val
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Asn Ile
739 740
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Ile His Asp
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Intron 15
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AT CAT TTT
His Phe
800 801
ACT CAA G gtaaaaattata.....
Thr Gln Asp
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AT GAT ATT
Asp Ile
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His Gln Arg
864 865 866
Intron 17
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A ATG AAA
Met Lys
867 868
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Phe Gln Lys
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Exon D
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Pro Glu Thr
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Lys Arg Leu
892 893 894
Exon D
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Intron 18
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Exon C
ATG CTT GAA
Met Leu Glu
892 893 894

Human OB Receptor Protein, Recombinant Secreted Receptor amino acid sequence (Seq. ID. No. 10):

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1    MICQKFCVVL LHWEFIYVIT AFNLSYPITP WRFKLSCMPP NSTYDYFLLP
51   AGLSKNTSNS NGHYETAVEP KFNSSGTHFS NLSKTTFHCC FRSEQDRNCS
101  LCADNIEGKT FVSTVNSLVF QQIDANWNIQ CWLKGDLKLF ICYVESLFKN
151  LFRNYNYKVH LLYVLPEVLE DSPLVPQKGS FQMVHCNCSV HECCECLVPV
201  PTAKLNDTLL MCLKITSGGV IFQSPLMSVQ PINMVKPDPP LGLHMEITDD
251  GNLKISWSSP PLVPFPLQYQ VKYSENSTTV IREADKIVSA TSLLVDSILP
301  GSSYEVQVRG KRLDGPGIWS DWSTPRVFTT QDVIYFPPKI LTSVGSNVSF
351  HCIYKKENKI VPSKEIVWWM NLAEKIPQSQ YDVVSDHVSK VTFFNLNETK
401  PRGKFTYDAV YCCNEHECHH RYAELYVIDV NINISCETDG YLTKMTCRWS
451  TSTIQSLAES TLQLRYHRSS LYCSDIPSIH PISEPKDCYL QSDGFYECIF
501  QPIFLLSGYT MWIRINHSLG SLDSPPTCVL PDSVVKPLPP SSVKAEITIN
551  IGLLKISWEK PVFPENNLQF QIRYGLSGKE VQWKMYEVYD AKSKSVSLPV
601  PDLCAVYAVQ VRCKRLDGLG YWSNWSNPAY TVVMDIKVPM RGPEFWRIIN
651  GDTMKKEKNV TLLWKPLMKN DSLCSVQRYV INHHTSCNGT WSEDVGNHTK
701  FTFLWTEQAH TVTVLAINSI GASVANFNLT FSWPMSKVNI VQSLSAYPLN
751  SSCVIVSWIL SPSDYKLMYF IIEWKNLNED GEIKWLRISS SVKKYYIHDH
801  FIPIEKYQFS LYPIFMEGVG KPKIINSFTQ DDIEKHQSD
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Human OB Receptor Protein, Recombinant Secreted Receptor DNA sequence (Seq. ID. No. 11):

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1     GCGGCCGCCA GTGTGATGGA TATCTGCAGA ATTCGGCTTT CTCTGCCTTC
51    GGTCGAGTTG GACCCCCGGA TCAAGGTGTA CTTCTCTGAA GTAAGATGAT
101   TTGTCAAAAA TTCTGTGTGG TTTTGTTACA TTGGGAATTT ATTTATGTGA
151   TAACTGGGTT TAACTTGTCA TATCCAATTA CTCCTTGGAG ATTTAAGTTG
201   TCTTGCATGC CACCAAATTC AACCTATGAC TACTTCCTTT TGCCTGCTGG
251   GCTCTCAAAG AATACTTCAA ATTCGAATGG ACATTATGAG ACAGCTGTTG
301   AACCTAAGTT TAATTCAAGT GGTACTCACT TTTCTAACTT ATCCAAAACA
351   ACTTTCCACT GTTGCTTTCG GAGTGAGCAA GATAGAAACT GCTCCTTATG
401   TGCAGACAAC ATTGAAGGAA AGACATTTGT TTCAACAGTA AATTCTTTAG
451   TTTTTCAACA AATAGATGCA AACTGGAACA TACAGTGCTG GCTAAAAGGA
501   GACTTAAAAT TATTCATCTG TTATGTGGAG TCATTATTTA AGAATCTATT
551   CAGGAATTAT AACTATAAGG TCCATCTTTT ATATGTTCTG CCTGAAGTGT
601   TAGAAGATTC ACCTCTGGTT GCCCAAAAAG GCAGTTTTCA GATGGTTCAC
651   TGCAATTGCA GTGTTCACGA ATGTTGTGAA TGTCTTGTGC CTGTGCCAAC
701   AGCCAAACTC AACGACACTC TCCTTATGTG TTTGAAAATC ACATCTGGTG
751   GAGTAATTTT CCAGTCACCT CTAATGTCAG TTCAGCCCAT AAATATGGTG
801   AAGCCTGATC CACCATTAGG TTTGCATATG GAAATCACAG ATGATGGTAA
851   TTTAAAGATT TCTTGGTCCA GCCCACCATT GGTACCATTT CCACTTCAAT
901   ATCAAGTGAA ATATTCAGAG AATTCTACAA CAGTTATCAG AGAAGCTGAC
951   AAGATTGTCT CAGCTACATC CCTGCTAGTA GACAGTATAC TTCCTGGGTC
1001  TTCGTATGAG GTTCAGGTGA GGGGCAAGAG ACTGGATGGC CCAGGAATCT
1051  GGAGTGACTG GAGTACTCCT CGTCTCTTTA CCACACAAGA TGTCATATAC
1101  TTTCCACCTA AAATTCTGAC AAGTGTTGGG TCTAATGTTT CTTTTCACTG
1151  CATCTATAAG AAGGAAAACA AGATTGTTCC CTCAAAAGAG ATTGTTTGGT
1201  GGATGAATTT AGCTGAGAAA ATTCCTCAAA GCCAGTATGA TGTTGTGAGT
```

1251 GATCATGTTA GCAAAGTTAC TTTTTTCAAT CTGAATGAAA CCAAACCTCG

1301 AGGAAAGTTT ACCTATGATG CAGTGTACTG CTGCAATGAA CATGAATGCC

1351 ATCATCGCTA TGCTGAATTA TATGTGATTG ATGTCAATAT CAATATCTCA

1401 TGTGAAACTG ATGGGTACTT AACTAAAATG ACTTGCAGAT GGTCAACCAG

1451 TACAATCCAG TCACTTGCGG AAAGCACTTT GCAATTGAGG TATCATAGGA

1501 GCAGCCTTTA CTGTTCTGAT ATTCCATCTA TTCATCCCAT ATCTGAGCCC

1551 AAAGATTGCT ATTTGCAGAG TGATGGTTTT TATGAATGCA TTTTCCAGCC

1601 AATCTTCCTA TTATCTGGCT ACACAATGTG GATTAGGATC AATCACTCTC

1651 TAGGTTCACT TGACTCTCCA CCAACATGTG TCCTTCCTGA TTCTGTGGTG

1701 AAGCCACTGC CTCCATCCAG TGTGAAAGCA GAAATTACTA TAAACATTGG

1751 ATTATTGAAA ATATCTTGGG AAAAGCCAGT CTTTCCAGAG AATAACCTTC

1801 AATTCCAGAT TCGCTATGGT TTAAGTGGAA AAGAAGTACA ATGGAAGATG

1851 TATGAGGTTT ATGATGCAAA ATCAAAATCT GTCAGTCTCC CAGTTCCAGA

1901 CTTGTGTGCA GTCTATGCTG TTCAGGTGCG CTGTAAGAGG CTAGATGGAC

1951 TGGGATATTG GAGTAATTGG AGCAATCCAG CCTACACAGT TGTCATGGAT

2001 ATAAAAGTTC CTATGAGAGG ACCTGAATTT TGGAGAATAA TTAATGGAGA

2051 TACTATGAAA AAGGAGAAAA ATGTCACTTT ACTTTGGAAG CCCCTGATGA

2101 AAAATGACTC ATTGTGCAGT GTTCAGAGAT ATGTGATAAA CCATCATACT

2151 TCCTGCAATG GAACATGGTC AGAAGATGTG GGAAATCACA CGAAATTCAC

2201 TTTCCTGTGG ACAGAGCAAG CACATACTGT TACGGTTCTG GCCATCAATT

2251 CAATTGGTGC TTCTGTTGCA AATTTTAATT TAACCTTTTC ATGGCCTATG

2301 AGCAAAGTAA ATATCGTGCA GTCACTCAGT GCTTATCCTT TAAACAGCAG

2351 TTGTGTGATT GTTTCCTGGA TACTATCACC CAGTGATTAC AAGCTAATGT

2401 ATTTTATTAT TGAGTGGAAA AATCTTAATG AAGATGGTGA AATAAAATGG

2451 CTTAGAATCT CTTCATCTGT TAAGAAGTAT TATATCCATG ATCATTTTAT

2501 CCCCATTGAG AAGTACCAGT TCAGTCTTTA CCCAATATTT ATGGAAGGAG

2551 TGGGAAAACC AAAGATAATT AATAGTTTCA CTCAAGATGA TATTGAAAAA

2601 CACCAGAGTG ATTGATAAGG ATCC

Human OB Receptor Protein, Recombinant Secreted Receptor DNA sequence with C-terminal FLAG (Seq. ID. No. 12)

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1     CCATTGAAGT CAATGGGAGT TTGTTTTGGC ACCAAAATCA ACGGGGATTT
51    CCAAAATGTC GTAATAACCC CGCCCCGTTG ACGCAAATGG GCGGTAGGCG
101   TGTACGGTGG GAGGTCTATA TAAGCAGAGC TCGTTTAGTG AACCGTCAGA
151   TCTCTAGAAG CTGGGTACCA GCTGCTAGCA AGCTTGCTAG CGGCCGCCAG
201   TGTGATGGAT ATCTGCAGAA TTCGGCTTTC TCTGCCTTCG GTCGAGTTGG
251   ACCCCCGGAT CAAGGTGTAC TTCTCTGAAG TAAGATGATT TGTCAAAAAT
301   TCTGTGTGGT TTTGTTACAT TGGGAATTTA TTTATGTGAT AACTGCGTTT
351   AACTTGTCAT ATCCAATTAC TCCTTGGAGA TTTAAGTTGT CTTGCATGCC
401   ACCAAATTCA ACCTATGACT ACTTCCTTTT GCCTGCTGGG CTCTCAAAGA
451   ATACTTCAAA TTCGAATGGA CATTATGAGA CAGCTGTTGA ACCTAAGTTT
501   AATTCAAGTG GTACTCACTT TTCTAACTTA TCCAAAACAA CTTTCCACTG
551   TTGCTTTCGG AGTGAGCAAG ATAGAAACTG CTCCTTATGT GCAGACAACA
601   TTGAAGGAAA GACATTTGTT TCAACAGTAA ATTCTTTAGT TTTTCAACAA
651   ATAGATGCAA ACTGGAACAT ACAGTGCTGG CTAAAAGGAG ACTTAAAATT
701   ATTCATCTGT TATGTGGAGT CATTATTTAA GAATCTATTC AGGAATTATA
751   ACTATAAGGT CCATCTTTTA TATGTTCTGC CTGAAGTGTT AGAAGATTCA
801   CCTCTGGTTC CCCAAAAAGG CAGTTTTCAG ATGGTTCACT GCAATTGCAG
851   TGTTCACGAA TGTTGTGAAT GTCTTGTGCC TGTGCCAACA GCCAAACTCA
901   ACGACACTCT CCTTATGTGT TTGAAAATCA CATCTGGTGG AGTAATTTTC
951   CAGTCACCTC TAATGTCAGT TCAGCCCATA AATATGGTGA AGCCTGATCC
1001  ACCATTAGGT TTGCATATGG AAATCACAGA TGATGGTAAT TTAAAGATTT
1051  CTTGGTCCAG CCCACCATTG GTACCATTTC CACTTCAATA TCAAGTGAAA
1101  TATTCAGAGA ATTCTACAAC AGTTATCAGA GAAGCTGACA AGATTGTCTC
1151  AGCTACATCC CTGCTAGTAG ACAGTATACT TCCTGGGTCT TCGTATGAGG
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1201 TTCAGGTGAG GGGCAAGAGA CTGGATGGCC CAGGAATCTG GAGTGACTGG

1251 AGTACTCCTC GTGTCTTTAC CACACAAGAT GTCATATACT TTCCACCTAA

1301 AATTCTGACA AGTGTTGGGT CTAATGTTTC TTTTCACTGC ATCTATAAGA

1351 AGGAAAACAA GATTGTTCCC TCAAAAGAGA TTGTTTGGTG GATGAATTTA

1401 GCTGAGAAAA TTCCTCAAAG CCAGTATGAT GTTGTGAGTG ATCATGTTAG

1451 CAAAGTTACT TTTTTCAATC TGAATGAAAC CAAACCTCGA GGAAAGTTTA

1501 CCTATGATGC AGTGTACTGC TGCAATGAAC ATGAATGCCA TCATCGCTAT

1551 GCTGAATTAT ATGTGATTGA TGTCAATATC AATATCTCAT GTGAAACTGA

1601 TGGGTACTTA ACTAAAATGA CTTGCAGATG GTCAACCAGT ACAATCCAGT

1651 CACTTGCGGA AAGCACTTTG CAATTGAGGT ATCATAGGAG CAGCCTTTAC

1701 TGTTCTGATA TTCCATCTAT TCATCCCATA TCTGAGCCCA AAGATTGCTA

1751 TTTGCAGAGT GATGGTTTTT ATGAATGCAT TTTCCAGCCA ATCTTCCTAT

1801 TATCTGGCTA CACAATGTGG ATTAGGATCA ATCACTCTCT AGGTTCACTT

1851 GACTCTCCAC CAACATGTGT CCTTCCTGAT TCTGTGGTGA AGCCACTGCC

1901 TCCATCCAGT GTGAAAGCAG AAATTACTAT AAACATTGGA TTATTGAAAA

1951 TATCTTGGGA AAAGCCAGTC TTTCCAGAGA ATAACCTTCA ATTCCAGATT

2001 CGCTATGGTT TAAGTGGAAA AGAAGTACAA TGGAAGATGT ATGAGGTTTA

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2101 TCTATGCTGT TCAGGTGCGC TGTAAGAGGC TAGATGGACT GGGATATTGG

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2201 TATGAGAGGA CCTGAATTTT GGAGAATAAT TAATGGAGAT ACTATGAAAA

2251 AGGAGAAAAA TGTCACTTTA CTTTGGAAGC CCCTGATGAA AAATGACTCA

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2351 AACATGGTCA GAAGATGTGG GAAATCACAC GAAATTCACT TTCCTGTGGA

2401 CAGAGCAAGC ACATACTGTT ACGGTTCTGG CCATCAATTC AATTGGTGCT

2451 TCTGTTGCAA ATTTTAATTT AACCTTTTCA TGGCCTATGA GCAAAGTAAA

2501 TATCGTGCAG TCACTCAGTG CTTATCCTTT AAACAGCAGT TGTGTGATTG

2551 TTTCCTGGAT ACTATCACCC AGTGATTACA AGCTAATGTA TTTTATTATT

2601 GAGTGGAAAA ATCTTAATGA AGATGGTGAA ATAAAATGGC TTAGAATCTC

2651 TTCATCTGTT AAGAAGTATT ATATCCATGA TCATTTTATC CCCATTGAGA

2701 AGTACCAGTT CAGTCTTTAC CCAATATTTA TGGAAGGAGT GGGAAAACCA

2751 AAGATAATTA ATAGTTTCAC TCAAGATGAT ATTGAAAAAC ACCAGAGTGA

2801 TGCAGGTGAC TACAAGGACG ACGATGACAA GTAGGGATCC AGACATGATA

2851 AGATACATTG ATGAGTTTGG ACAACCCACA ACTAGAATGC AGTGAAAAAA

2901 ATGCTTTATT TGTGAAATTT GTGATGCTAT TGCTTTATTT GTAACCAT

Recombinant Human OB Receptor Protein, Natural Splice Variant amino acid sequence (Seq. ID. No. 13)

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1    MICQKFCVVL LHWEFIYVIT AFNLSYPITP WRFKLSCMPP NSTYDYFLLP
51   AGLSKNTSNS NGHYETAVEP KFNSSGTHFS NLSKTTFHCC FRSEQDRNCS
101  LCADNIEGKT FVSTVNSLVF QQIDANWNIQ CWLKGDLKLF ICYVESLFKN
151  LFRNYNYKVH LLYVLPEVLE DSPLVPQKGS FQMVHCNCSV HECCECLVPV
201  PTAKLNDTLL MCLKITSGGV IFQSPLMSVQ PINMVKPDPP LGLHMEITDD
251  GNLKISWSSP PLVPFPLQYQ VKYSENSTTV IREADKIVSA TSLLVDSILP
301  GSSYEVQVRG KRLDGPGIWS DWSTPRVFTT QDVIYFPPKI LTSVGSNVSF
351  HCIYKKENKI VPSKEIVWWM NLAEKIPQSQ YDVVSDHVSK VTFFNLNETK
401  PRGKFTYDAV YCCNEHECHH RYAELYVIDV NINISCETDG YLTKMTCRWS
451  TSTIQSLAES TLQLRYHRSS LYCSDIPSIH PISEPKDCYL QSDGFYECIF
501  QPIFLLSGYT MWIRINHSLG SLDSPPTCVL PDSVVKPLPP SSVKAEITIN
551  IGLLKISWEK PVFPENNLQF QIRYGLSGKE VQWKMYEVYD AKSKSVSLPV
601  PDLCAVYAVQ VRCKRLDGLG YWSNWSNPAY TVVMDIKVPM RGPEFWRIIN
651  GDTMKKEKNV TLLWKPLMKN DSLCSVQRYV INHHTSCNGT WSEDVGNHTK
701  FTFLWTEQAH TVTVLAINSI GASVANFNLT FSWPMSKVNI VQSLSAYPLN
751  SSCVIVSWIL SPSDYKLMYF IIEWKNLNED GEIKWLRISS SVKKYYIHGK
801  FTIL
```

Human OB Receptor Protein, Natural Splice Variant DNA (Seq. ID. No. 14)

```
1     GCGGCCGCCA GTGTGATGGA TATCTGCAGA ATTCGGCTTT CTCTGCCTTC
51    GGTCGAGTTG GACCCCCGGA TCAAGGTGTA CTTCTCTGAA GTAAGATGAT
101   TTGTCAAAAA TTCTGTGTGG TTTTGTTACA TTGGGAATTT ATTTATGTGA
151   TAACTGCGTT TAACTTGTCA TATCCAATTA CTCCTTGGAG ATTTAAGTTG
201   TCTTGCATGC CACCAAATTC AACCTATGAC TACTTCCTTT TGCCTGCTGG
251   GCTCTCAAAG AATACTTCAA ATTCGAATGG ACATTATGAG ACAGCTGTTG
301   AACCTAAGTT TAATTCAAGT GGTACTCACT TTTCTAACTT ATCCAAAACA
351   ACTTTCCACT GTTGCTTTCG GAGTGAGCAA GATAGAAACT GCTCCTTATG
401   TGCAGACAAC ATTGAAGGAA AGACATTTGT TTCAACAGTA AATTCTTTAG
451   TTTTTCAACA AATAGATGCA AACTGGAACA TACAGTGCTG GCTAAAAGGA
501   GACTTAAAAT TATTCATCTG TTATGTGGAG TCATTATTTA AGAATCTATT
551   CAGGAATTAT AACTATAAGG TCCATCTTTT ATATGTTCTG CCTGAAGTGT
601   TAGAAGATTC ACCTCTGGTT CCCCAAAAAG GCAGTTTTCA GATGGTTCAC
651   TGCAATTGCA GTGTTCACGA ATGTTGTGAA TGTCTTGTGC CTGTGCCAAC
701   AGCCAAACTC AACGACACTC TCCTTATGTG TTTGAAAATC ACATCTGGTG
751   GAGTAATTTT CCAGTCACCT CTAATGTCAG TTCAGCCCAT AAATATGGTG
801   AAGCCTGATC CACCATTAGG TTTGCATATG GAAATCACAG ATGATGGTAA
851   TTTAAAGATT TCTTGGTCCA GCCCACCATT GGTACCATTT CCACTTCAAT
901   ATCAAGTGAA ATATTCAGAG AATTCTACAA CAGTTATCAG AGAAGCTGAC
951   AAGATTGTCT CAGCTACATC CCTGCTAGTA GACAGTATAC TTCCTGGGTC
1001  TTCGTATGAG GTTCAGGTGA GGGGCAAGAG ACTGGATGGC CCAGGAATCT
1051  GGAGTGACTG GAGTACTCCT CGTGTCTTTA CCACACAAGA TGTCATATAC
1101  TTTCCACCTA AAATTCTGAC AAGTGTTGGG TCTAATGTTT CTTTTCACTG
1151  CATCTATAAG AAGGAAAACA AGATTGTTCC CTCAAAAGAG ATTGTTTGGT
1201  GGATGAATTT AGCTGAGAAA ATTCCTCAAA GCCAGTATGA TGTTGTGAGT
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1251 GATCATGTTA GCAAAGTTAC TTTTTTCAAT CTGAATGAAA CCAAACCTCG

1301 AGGAAAGTTT ACCTATGATG CAGTGTACTG CTGCAATGAA CATGAATGCC

1351 ATCATCGCTA TGCTGAATTA TATGTGATTG ATGTCAATAT CAATATCTCA

1401 TGTGAAACTG ATGGGTACTT AACTAAAATG ACTTGCAGAT GGTCAACCAG

1451 TACAATCCAG TCACTTGCGG AAAGCACTTT GCAATTGAGG TATCATAGGA

1501 GCAGCCTTTA CTGTTCTGAT ATTCCATCTA TTCATCCCAT ATCTGAGCCC

1551 AAAGATTGCT ATTTGCAGAG TGATGGTTTT TATGAATGCA TTTTCCAGCC

1601 AATCTTCCTA TTATCTGGCT ACACAATGTG GATTAGGATC AATCACTCTC

1651 TAGGTTCACT TGACTCTCCA CCAACATGTG TCCTTCCTGA TTCTGTGGTG

1701 AAGCCACTGC CTCCATCCAG TGTGAAAGCA GAAATTACTA TAAACATTGG

1751 ATTATTGAAA ATATCTTGGG AAAAGCCAGT CTTTCCAGAG AATAACCTTC

1801 AATTCCAGAT TCGCTATGGT TTAAGTGGAA AAGAAGTACA ATGGAAGATG

1851 TATGAGGTTT ATGATGCAAA ATCAAAATCT GTCAGTCTCC CAGTTCCAGA

1901 CTTGTGTGCA GTCTATGCTG TTCAGGTGCG CTGTAAGAGG CTAGATGGAC

1951 TGGGATATTG GAGTAATTGG AGCAATCCAG CCTACACAGT TGTCATGGAT

2001 ATAAAAGTTC CTATGAGAGG ACCTGAATTT TGGAGAATAA TTAATGGAGA

2051 TACTATGAAA AAGGAGAAAA ATGTCACTTT ACTTTGGAAG CCCCTGATGA

2101 AAAATGACTC ATTGTGCAGT GTTCAGAGAT ATGTGATAAA CCATCATACT

2151 TCCTGCAATG GAACATGGTC AGAAGATGTG GGAAATCACA CGAAATTCAC

2201 TTTCCTGTGG ACAGAGCAAG CACATACTGT TACGGTTCTG GCCATCAATT

2251 CAATTGGTGC TTCTGTTGCA AATTTTAATT TAACCTTTTC ATGGCCTATG

2301 AGCAAAGTAA ATATCGTGCA GTCACTCAGT GCTTATCCTT TAAACAGCAG

2351 TTGTGTGATT GTTTCCTGGA TACTATCACC CAGTGATTAC AAGCTAATGT

2401 ATTTTATTAT TGAGTGGAAA AATCTTAATG AAGATGGTGA AATAAAATGG

2451 CTTAGAATCT CTTCATCTGT TAAGAAGTAT TATATCCATG GTAAGTTTAC

2501 TATACTT

While the present invention has been described in terms of preferred embodiments, it is understood that variations and modifications will occur to those skilled in the art. Therefore, it is intended that the appended claims cover all such equivalent variations which come within the scope of the invention as claimed.

CLAIMS

1. An OB receptor protein preparation containing an OB receptor protein, optionally in a pharmaceutically acceptable formulation, said OB receptor protein having part or all of the amino acid sequence according to Seq. ID No. 1 and one or more of the biological properties of naturally occurring OB receptor protein.

2. An OB receptor protein preparation containing an OB receptor protein, optionally in a pharmaceutically acceptable formulation, wherein said OB receptor protein amino acid sequence is selected from among amino acid sequences (according to Seq. ID No. 1):

(a) 1-896;

(b) 22-896 optionally with an N-terminal methionyl residue;

(c) 23-896 optionally with an N-terminal methionyl residue;

(d) 29-896 optionally with an N-terminal methionyl residue;

(e) 1-839;

(f) 22-839 optionally with an N-terminal methionyl residue;

(g) 29-839 optionally with an N-terminal methionyl residue;

(h) 1-841;

(i) 22-841 optionally with an N-terminal methionyl residue;

(j) 23-841 optionally with an N-terminal methionyl residue;

(k) 29-841 optionally with an N-terminal methionyl residue;

(l) 1-891;

(m) 22-891 optionally with an N-terminal methionyl residue;

(n) 23-891 optionally with an N-terminal methionyl residue;

(o) 29-891 optionally with an N-terminal methionyl residue;

(p) of subparts (l) through (o) further having the C-terminal amino acids, beginning at position 892, of OB receptor B (Seq. ID No. 3) or C (Seq. ID. No. 5); and,

(q) a chemically modified derivative of any of subparts (a) through (p).

3. An OB receptor protein preparation of claim 2 wherein said OB receptor protein is further selected from among the OB receptor proteins of subparts (l) through (o) further having the C-terminal amino acids, beginning at position 892, of OB receptor protein D (Seq. ID No. 7).

4. An OB receptor protein preparation of claim 2 wherein said OB receptor protein is further selected from among the OB receptor proteins of subparts (l) through (o) further having substituted the C-terminal amino acids, beginning at position 799, G K F T I L (Seq. ID No. 13).

5. An OB receptor protein preparation according to any of claims 1 through 4, wherein the extracellular domain of said OB receptor protein is modified, said modification selected from among:

(a) deletion of all or part of the random coil domain;

(b) modification of one or both "WSXWS" boxes by substition of the first serine with another amino acid;

(c) modification of one or both "WSXWS" boxes by substitution of the last serine with another amino acid; and

(d) modification of one or both "WSXWS" boxes by substitution of the first tryptophan with another amino acid.

6. A DNA molecule encoding an OB receptor protein according to any of claims 1-5 selected from the group consisting of:

(a) the DNA sequences set forth in Seq. ID nos. 2, 4, 6, 8, 11, 12, and 14;

(b) a DNA which selectively hybridizes to a DNA of subpart (a); and

(c) a DNA which, but for the degeneracy of the genetic code would hybridize to a DNA of subpart (a) or (b).

7. A biologically functional viral or plasmid vector containing a DNA of claim 6.

8. A procaryotic or eucaryotic host cell containing the vector of claim 7.

9. A host cell modified so that expression of endogenous OB receptor protein is enhanced.

10. A host cell of claim 9 which is an isolated human host cell.

11. A process for producing an OB receptor protein comprised of culturing, under suitable conditions, a host cell according to any of claims 8, 9 or 10, obtaining the OB receptor produced, and optionally preparing a pharmaceutical composition containing said OB receptor.

12. A method of treating an individual for a therapeutic disorder selected from among obesity, diabetes, high blood lipid levels, and high cholesterol levels comprised of administering a therapeutic amount of an OB receptor protein preparation containing an OB receptor protein according to any of claims 1-5, or produced by the process according to claim 11.

13. A method of treating an individual for weight loss or weight maintenance for solely cosmetic purposes comprised of administering an effective amount of an OB receptor preparation containing an OB receptor protein according to any of claims 1-5, or produced by the process according to claim 11.

14. Use of an OB receptor protein according to claims 1-5, or produced by the process of claim 11, for manufacturing a medicament for the treatment of obesity, diabetes, high blood lipid levels, or high cholesterol levels.

15. An OB protein/OB receptor protein complex preparation, containing an OB protein moiety and an OB receptor protein moiety, optionally in a pharmaceutically acceptable formulation, wherein:

(a) said OB receptor protein is selected from among those set forth in any of claims 1 and 2;

(b) said OB protein moiety is selected from among:

(i) a naturally ocurring OB protein; and,

(ii) a non-naturally ocurring OB protein, analog or derivative thereof.

16. An OB protein/OB receptor protein complex preparation of claim 15 wherein said OB receptor protein is selected from among those set forth in any of claims 3, 4, and 5.

17. A method of treating an individual for a therapeutic disorder selected from among obesity, diabetes, high blood lipid levels, and high cholesterol levels comprised of administering a therapeutic amount of an OB protein/OB receptor protein complex preparation of claims 15 or 16.

18. A method of claim 17 wherein said OB protein/OB receptor protein complex preparation is formed in vivo by administering, into a patient, a first population of cells expressing an OB protein, and a second population of cells expressing an OB receptor protein.

19. A method of treating an individual for weight loss or weight maintenance for solely cosmetic purposes comprised of administering a therapeutic amount of an OB protein/OB receptor protein complex preparation containing an OB receptor protein moiety according to any of claims 1-5, or produced by the process according to claim 11.

20. Use of an OB protein/OB receptor protein complex preparation, according to claims 15 or 16, for manufacturing a medicament for the treatment of obesity, diabetes, high blood lipid levels, or high cholesterol levels.